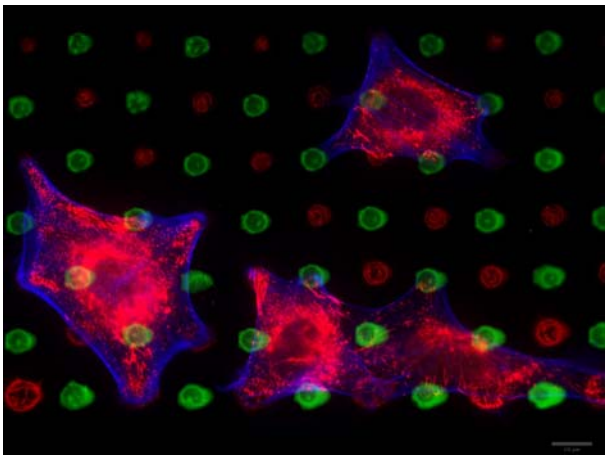
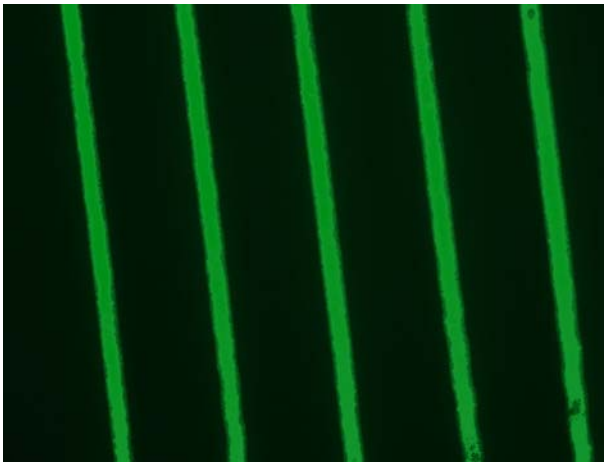
Nano eNabler™ System

BioForce Nanosciences, Inc.
Nano eNabler™ System
User Manual Version 1.2





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1 Introduction

1.1 Welcome to the Nano eNabler™ System

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1.1 Welcome to the Nano eNabler™ System

The Nano eNabler™ System is the most powerful surface patterning technology available for dispensing attoliter to femtoliter volumes of DNA, proteins, and other molecules. These features, which typically measure between 1-20 microns, can be printed onto a wide variety of surfaces. The Nano eNabler™ is an instrument platform consisting of supporting hardware components, an easy-to-use software controller interface, and an array of tools that permit real-time observation of the printing process. The Nano eNabler™ System can play a key role in the experimental design process in your laboratory. Research and diagnostics alike will benefit from rapid application development. The Nano eNabler™ System combines speed, precision, and flexibility to bring surface patterning to a new level.

1.2 Nano eNabler™ Key Features

The Nano eNabler™ System is a tool to aid researchers in printing minute volumes of liquids onto solid surfaces. The Nano eNabler™ System emphasizes a rapid, precise, and advanced approach to the deposition of materials such as DNA or proteins for diagnostics or research development. Advances in sample deposition are made possible using a proprietary approach. The Nano eNabler™ System provides an unparalleled tool for genomics and proteomics research, diagnostics and testing.

1.2.1 The Nano eNabler™

- Power Requirements: 120/240 VAC, 10 Amps
- Dimensions: 51 cm x 37 cm x 33 cm
- Controller Dimensions: 54 cm x 54 cm x 64 cm
- Instrument Weight: 18.14 kg (40 lb)
- Controller Weight: 38.6 kg (85 lb)

- X,Y Stage Travel Range: 50 mm x 50 mm
- X,Y Stage Resolution: 20 nm
- Z Stage Range: 45 mm
- Z Stage Fine Resolution: 100 nm
- Controllable Humidity Range: 25 - 80% RH
- Motorized Optical Microscope (150X to 1000X) with Integrated Video Capture
- Pentium 4 3.0GHz, 512MB
- Windows XP Professional

1.2.2 NanoWare™ Software

The Nano eNabler™ System utilizes proprietary BioForce software that allows the end user to control every aspect of the deposition process.

1.3 The Scope of This Manual

This manual attempts to explain how to use The Nano eNabler™ System. We recognize that the fields of molecular biology and nanotechnology are still rapidly developing areas and the tools and methodologies used are still emerging. We have endeavored to make the text as accessible as possible to scientists of different backgrounds, however, certain assumptions are made:

1.3.1 Limitations of This Manual

1. This manual does not cover basics that can be found in standard biochemistry text books. The reader is assumed to have some knowledge of the nature of DNA, proteins or other desired chemistries.
2. Users are expected to be comfortable with the use of computers and graphical user interfaces. Menus, scrollbars, point and click/double-click, moving and resizing windows should all be familiar concepts and are referred to without explanation.
3. This manual is not a molecular biology text book. Where necessary, basic descriptions of specific tools and methods are included in this document for clarity. These discussions, however, are necessarily brief.

1.4 How to Use This Manual

This book introduces The Nano eNabler™ System. Chapter 2 Basic Concepts provides an introduction to some basic concepts of the precision and control of the Nano eNabler™, DNA and protein sample preparation, and safety concerns in the context of the Nano eNabler™ System. Chapter 3: The Nano eNabler™ Instrumentation – The Nano eNabler™, introduces the hardware components. Chapter 4: The Nano eNabler™ Software – The Graphical User Interface introduces the control software for the Nano eNabler™ for the controlled deposition of target domains. The primary aim of this approach is to introduce both the functionality and the utility of the Nano eNabler™ instrumentation while demonstrating how to use the graphical user interface.

The manual is split into chapters and the chapters into sections. Both are listed in the table of contents. From time to time, you will also see margin notes. Please read these notes, as they will usually contain information about what to watch out for when working through a section. Instruction on how to get to a particular window, or execute a certain function will be given in step-by-step format with accompanying figures. All new figures are labeled and broken down into their constituent panes when necessary. Examples of the instrument's basic and alternative configurations will be given as well as some inferences that can be made about the changes when applied. This information will then be built upon as additional features are explained.

1.4.1 Documentation User Attention Words

Several user attention words or symbols appear in this manual. Each word implies a particular level of observation or action as described below:
NOTE: Calls attention to useful information.

IMPORTANT: Indicates information that is necessary for proper instrument operation.

CAUTION: Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING: Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

DANGER: Indicates an imminently hazardous situation which, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

1.5 Reader Feedback

If you have an idea or suggestion on how we can improve this document, please let us know. Please direct communications to the postal address given inside the front cover of this book or email us at websupport@bioforcenano.com

2 Basic Concepts

2.1 System Requirements

2.2 Precautions and Hazards

2.3 DNA and Protein Arrays

2.4 X-Y Precise Position Control

2.5 Force Feedback for Z-Position Control

2.6 Environmental Control

2.7 Surface Preparation

2.8 Sample Preparation

2.9 Surface Patterning Tool Selection and Preparation

2.10 The Nano eNabler™ and NanoWare™ Software

2.1 System Requirements

2.1.1 Operating System and Computer Hardware

The Nano eNabler™ utilizes an integrated computer system. No external computer is necessary.

2.1.2 Electrical

Power Requirements: 120/240 VAC, 10 Amps

2.1.3 Environmental

The laboratory temperature should be maintained between 15–30°C (59– 85°F). The instrument can tolerate up to 80% relative humidity. Avoid placing the instrument adjacent to heaters or cooling ducts.

2.1.4 Vibration

For best results, place the Nano eNabler™ on a bench top or sturdy desk/table capable of supporting at least 40 lbs. No additional vibration isolation equipment is necessary for typical usage. Avoid placing the instrument in close proximity to vacuum pumps, large motors, and other sources of vibration.

2.2 Precautions and Hazards

2.2.1 Electrical

WARNING: ELECTRICAL SHOCK WARNING. Risk of electric shock. Disconnect power cord from supply before replacing fuses or removing power supply module from instrument. Replacement or inspection of any components should be carried out only by experienced service personnel. It is important to completely power down the Nano eNabler before unplugging or plugging in components. The power connector integrated into the camera is susceptible to shorting out and restarting the system if plugged/unplugged at an angle.

2.2.2 Mechanical

WARNING: MECHANICAL HAZARD. Potential for cuts, abrasions, or other minor bodily injury. Some edges may be sharp, particularly those of the Environmental Control Chamber. Avoid contact with sharp edges. Keep hands out of the path of X, Y, and Z stage travel while the instrument is in operation. If you must reach inside the Environmental Control Chamber, ensure that all instrument motion has stopped and that no additional software buttons are pressed until your hands have been safely removed from the Environmental Control Chamber.

2.2.3 Chemical

WARNING: CHEMICAL HAZARD. Some of the chemicals used in the course of sample preparation or deposition may be potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (e.g., safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Do not leave chemical containers open. Use only with adequate ventilation.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

WARNING: CHEMICAL WASTE HAZARD. Wastes produced by methods for the preparation and deposition of DNA, proteins, or other chemicals are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDS) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.

- Handle chemical wastes in a fume hood.
- Minimize contact with and inhalation of chemical waste. Wear appropriate personal protective equipment when handling chemicals (*e.g.*, safety glasses, gloves, or protective clothing).
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

IMPORTANT: ABOUT WASTE DISPOSAL. As the generator of potentially hazardous waste, it is your responsibility to perform the actions listed below:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, or national regulations.

NOTE: Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

2.2.4 Laser

WARNING: LASER HAZARD. Exposure to direct or redirected laser light can burn the retina and leave permanent blind spots. Never look directly into the laser beam. Remove jewelry and anything else that can redirect the beam into your eyes. Wear laser safety goggles during laser alignment. Protect others from exposure to the beam. Post a laser warning sign while performing the alignment.

WARNING: LASER HAZARD. When instrument panels are removed, laser light may be evident. Qualified service personnel should wear laser safety goggles when removing panels for service.

2.2.5 Environmental

IMPORTANT: ALTITUDE. This instrument is for indoor use only and not for excessive altitudes.

IMPORTANT: TEMPERATURE AND HUMIDITY. The laboratory temperature should be maintained between 15–30°C (59– 85°F). The instrument can tolerate up to 80% relative humidity. Avoid placing the instrument adjacent to heaters or cooling ducts.

IMPORTANT: POLLUTION. The installation category (over voltage category) for this instrument is II, and it is classified as portable equipment. The instrument has a pollution degree rating of 2, and may be installed in an environment that has non-conductive pollutants only.

IMPORTANT: HEAT. The typical thermal output of the instrument is low. Consult your facilities department regarding ventilation requirements for this level of heat output. The

control system is equipped with a fan for the dissipation of heat generated by the electronic components.

2.2.6 Ergonomical

WARNING: PHYSICAL INJURY HAZARD.

Moving and Lifting the Instrument — Improper lifting can cause painful and sometimes permanent back injury. Use proper lifting techniques when lifting or moving the instrument. Safety training for proper lifting techniques is recommended. Do not attempt to lift or move the instrument without the assistance of others.

CAUTION: MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD.

These hazards are caused by the following potential risk factors which include, but are not limited to, repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.
Safe and Efficient Computer Use—Operating the computer correctly prevents stress-producing effects such as fatigue, pain, and strain.
To minimize these effects on your back, legs, eyes, and upper extremities (neck, shoulder, arms, wrists, hands and fingers), design your workstation to promote neutral or relaxed working positions. This includes working in an environment where heating, air conditioning, ventilation, and lighting are set correctly. See the guidelines below:

- Use a seating position that provides the optimum combination of comfort, accessibility to the keyboard, and freedom from fatigue-causing stresses and pressures.
- The bulk of the person's weight should be supported by the buttocks, not the thighs.
- Feet should be flat on the floor, and the weight of the legs should be supported by the floor, not the thighs.
- Lumbar support should be provided to maintain the proper concave curve of the spine.
- Place the keyboard on a surface that provides:
    - The proper height to position the forearms horizontally and upper arms vertically.
    - Support for the forearms and hands to avoid muscle fatigue in the upper arms.
    - Position the viewing screen to the height that allows normal body and head posture. This height depends upon the physical proportions of the user.
- Adjust vision factors to optimize comfort and efficiency by:
    - Adjusting screen variables, such as brightness, contrast, and color, to suit personal preferences and ambient lighting.
    - Positioning the screen to minimize reflections from ambient light sources.
    - Positioning the screen at a distance that takes into account user variables such as nearsightedness, farsightedness, astigmatism, and the effects of corrective lenses.

- When considering the user's distance from the screen, the following are useful guidelines:

  - The distance from the user's eyes to the viewing screen should be approximately the same as the distance from the user's eyes to the keyboard.
  - For most people, the reading distance that is the most comfortable is approximately 20 inches.
  - The workstation surface should have a minimum depth of 36 inches to accommodate distance adjustment.
  - Adjust the screen angle to minimize reflection and glare, and avoid highly reflective surfaces for the workstation.

- Use a well-designed copy holder, adjustable horizontally and vertically, that allows referenced hard-copy material to be placed at the same viewing distance as the screen and keyboard.
- Keep wires and cables out of the way of users and passersby.
- Choose a workstation that has a surface large enough for other tasks and that provides sufficient legroom for adequate movement.

2.3 Applications

The Nano eNabler System is a powerful tool which can be applied to a wide variety of applications at the micro and nanoscale. Although three of the most popular application groups are briefly discussed below, these are by no means meant to limit the creativity of the end user.

2.3.1 Molecular Detection

The field of molecular detection methodologies encompasses chemical and biological sensors as well as lab-on-a-chip devices. These sensing technologies can often benefit from the liquid dispensing capabilities of the Nano eNabler for functionalization or construction of the detection mechanism. Additional applications include BioMEMS functionalization, and printing biomolecular arrays inside microfluidic channels that are narrower than possible with conventional spotting techniques.

2.3.2 Diagnostics and Pharmaceutical Discovery

Biomolecular arrays can be useful tools for both research and diagnostics. Typically, a series of ssDNA probes or affinity capture molecules such as an antibody are patterned onto a surface that possesses the proper chemistry for robust immobilization. These affinity agents are then exposed to a sample of interest, with a reporter system utilized to identify binding events. Standard arrays can consume large amounts of sample, whereas ultraminiaturized arrays constructed with the Nano eNabler can be used with 1 µl or less. This reduction in volume paves the way for small volume biomarker analysis from Laser Capture Microdissection (LCM) samples, small animal/organism model systems, pre-natal samples, and even single cells.

Biomolecular arrays constructed with the Nano eNabler can have applications other than biomarker analysis. Cell adhesion molecules or signaling proteins can be printed onto a surface that is suitable for cell culture. As the cells interact with protein domains that are smaller than a single cell, valuable information can be gathered about stem cell differentiation or proliferation, cell motility and chemotaxis, interactions of the immune system, as well as scaffolds for tissue engineering.

2.3.3 Engineering Surface Architectures

The third major category of Nano eNabler applications is less oriented towards biology and more towards nanotechnology and materials science. Nanomaterials such as quantum dots, colloids, and magnetic nanoparticles can all be directly printed onto a surface. This capability may be important for device construction or analysis. Other materials that may be useful for surface modification can be directly deposited, including etchants, resists, and adhesives.

2.4 X-Y Precision Motion Control

The Nano eNabler™ has a high resolution piezo driven X-Y translation stage with 50 mm of travel in each direction. This stage system uses optical linear encoders to track stage movements. The stage encoder system allows precision moves with 20 nm of resolution. However, errors in the mechanical system limit practical resolutions to about 100 nm over short moves (less than 1 inch or 25 mm). Moves of longer distances will result in less precise X-Y Stage system moves.

Most moves will be software-based resulting from the user interacting with the software. These moves can be the result of a user pressing a “Move” button displayed on screen, entering a destination in the X-Y Go To command, by automated moves to saved locations, or those that result from calculations of the software as it constructs a user defined array. Each of these types of moves will be discussed in greater detail in the “software” section later in this manual.

2.5 Force Feedback for Z-Position Control

2.5.1 Using Lasers

The Nano eNabler™ uses a laser beam to track the position of the Surface Patterning Tool (SPT). While precautions should always be taken when working with laser beams, the power of the Nano eNabler™ laser is very low (0.9 mW)

WARNING: LASER HAZARD. Exposure to direct or redirected laser light can burn the retina and leave permanent blind spots. Never look directly into the laser beam. Remove jewelry and anything else that can redirect the beam into your eyes. Wear laser safety goggles during laser alignment. Protect others from exposure to the beam. Post a laser warning sign while performing the alignment.

WARNING: LASER HAZARD. When instrument panels are removed, laser light may be evident. Wear laser safety goggles when you remove panels for service.

2.5.2 The Optical Lever

An optical lever is a widely used method of monitoring deflections of a surface. Laser light from a solid state diode is directed to and reflected off the back of a microfabricated Surface Patterning Tool (SPT) and collected by a position sensitive photodetector (PSD) consisting of two closely spaced photodiodes whose output signal is collected by a differential amplifier. In this instance, the “lever” part of the optical lever refers not to the SPT but to the displacement amplification provided by the length of the laser beam’s travel.

The laser reflection path is initially directed to the center of the split photodetector such that each of the two parts receives an equal half of the total reflected light. Angular displacement of the SPT upon contact with a surface results in one photodiode collecting more light than the other photodiode, resulting in an output signal (the difference between the photodiode signals normalized by their sum) which is proportional to the deflection of the cantilever.

2.5.3 Sum and Difference

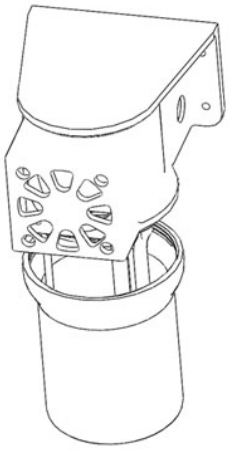
Two types of information result from the monitoring of the optical lever. They are the Sum and the Difference. The Sum value is the total amount of light striking both halves of the photodetector. The Difference value is the difference between the two photodiode signals, normalized by their Sum.

Rapid changes to the sum and/or difference value are indicative of an angular deflection of the SPT resulting from a contact event with the surface.

2.6 Environmental Control

Humidity is one of the most important variables to control when attempting to print micron or sub-micron scale features. Insufficient humidity can slow or prevent material transfer, and excessive humidity can negatively affect regulation of feature size. The Nano eNabler™ is equipped with an outer enclosure that provides a barrier between the Nano eNabler™ instrumentation and the ambient room conditions.

The Nano eNabler™ environmental control chamber allows complete control of the humidity surrounding the instrument. The front door accommodates full access for initial setup or periodic adjustments. Desired humidity is set through the NanoWare™ software interface and achieved using a computer-controlled system of dry gas and humid air flow. Dry conditions are attained by filling the chamber with a user-supplied inert dry gas such as nitrogen. Be sure to specify that you need a high quality dry gas when you order the tank from your gas supplier, as some lower grades of nitrogen contain water and other impurities. Humid environmental conditions are realized by the humidification device attached to the environmental chamber which creates a mist of $ddiH_2O$. The screw-on cup should be checked periodically to ensure that the misting device is completely submerged in clean $ddiH_2O$. If the water appears cloudy or discolored, you should wash the cup thoroughly with a strong detergent, rinse well, and refill with $ddiH_2O$. We recommend cleaning the cup and replacing the water at least once each week to limit microbial growth.



Humidity must be precisely controlled to allow optimal loading and unloading of materials onto and off of the SPT. Insufficient humidity can prevent adequate loading of materials onto the SPT and inhibit proper transfer onto the chip surface. Conversely, too much humidity can lead to difficulties in regulating spot size. Capillary action will tend to pull larger amounts of fluid from the microfluidic channel onto the surface if the humidity is too high.

2.7 Surface Preparation

The Nano eNabler™ can accommodate a wide variety of surfaces for deposition. The process is governed by principles similar to those common in the microarraying realm. As such, nearly all of the surface treatments and chemistries utilized for microarraying are compatible with the Nano eNabler™ platform. This includes both two-dimensional and three-dimensional chemistries, although only the two-dimensional surfaces should be used for height-based assays using an atomic force microscope (AFM). The deposition process is optically monitored from above, allowing both transparent substrates such as glass, and opaque substrates such as silicon or metal-coated materials. While traditional microarrayers generally utilize 1" x 3" glass slides, the Nano eNabler™ format is miniaturized down to a more efficient 4 mm x 4 mm indexed substrate. Indexed silicon and glass substrates (Sindex™chips) are available from BioForce Nanosciences either without any surface treatments, or with a variety of standard chemistries. Contact sales@bioforcenano.com for additional details.

2.7.1 Surface preparation for DNA

Suitable surface treatments for deposition of DNA arrays include poly-l-lysine and amino-silanes for electrostatic binding of unmodified DNA. Amino-modified DNA can be covalently tethered to standard amino-reactive surfaces such as epoxy-silanes, aldehyde-silanes, NHS-esters, or a variety of three-dimensional polymer matrices. There are also a number of commercially available proprietary chemistries available from other vendors.

NOTE: Surfaces containing hydrolysable functional groups may lose binding efficiency after extended periods at high humidity in the Nano eNabler™ environmental control chamber.

2.7.2 Surface preparation for Proteins

Protein arrays for fluorescent assay applications are preferentially constructed on reactive three-dimensional polymer matrix surfaces. These generally have a higher protein binding capacity, and tend to maintain protein hydration and conformation for optimal activity. For AFM-based protein interaction assays, it is important to select a two-dimensional chemistry that offers covalent attachment with minimal surface roughness. BioForce Nanosciences has engineered an ideal solution to these strict requirements with ProLinker Sindex™ Chips. Antibody-based capture assays can be improved with proper antibody orientation using ProLinker Sindex™ Chips that have been pre-treated with Protein A/G.

2.8 Sample Preparation

The Nano eNabler™ can be used to print spots and lines of both organic and inorganic molecules on a wide variety of surfaces, and its applications are limited only by the imagination of the user. It was conceived and built as a powerful research-grade instrument with that goal in mind. Therefore, we can provide starting points and suggestions for deposition conditions, however we simply cannot anticipate every application or circumstance that our users may encounter. It must ultimately be left up to the end-user to determine the optimal sample preparation and conditions for their particular experiments.

2.8.1 Sample preparation for DNA

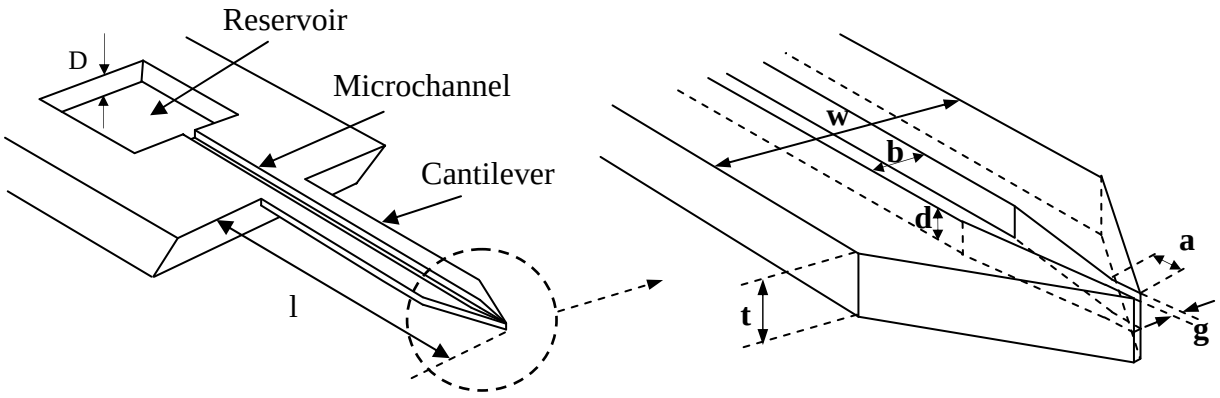
Mix your DNA sample at 0.1 mg/ml in 1x PBS with BioForce DNA Spotting Buffer at a 1:1 ratio. DNA concentrations of 1 mg/ml or higher may result in a sticky and highly viscous solution that is not suitable for deposition.

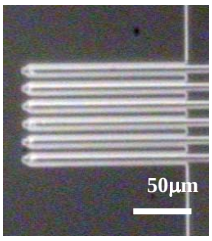
2.8.2 Sample preparation for Protein

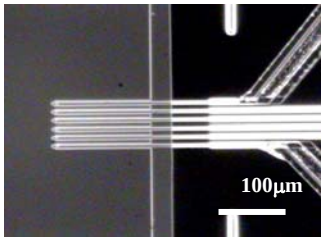
Mix your protein sample at 1mg/ml in 1x PBS with BioForce Protein Spotting Buffer at a 1:1 ratio. This formula has been painstakingly optimized for our printing process and allows binding to a wide variety of surfaces. It is crucial to avoid all primary amine-containing buffers such as Tris or glycine when spotting onto amine-reactive surfaces.

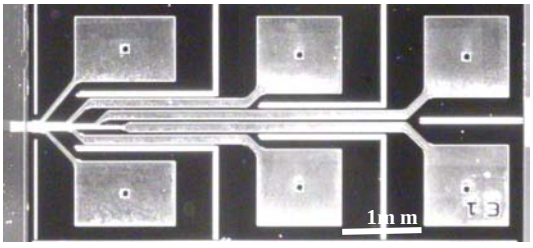
2.9 Surface Patterning Tool Selection and Preparation

Surface Patterning Tools (SPTs) are available in several designs and configurations; however the underlying principle is the same for all designs. The SPT is a long, thin silicon dioxide microcantilever jutting out from the edge of a silicon substrate. In contrast to a vertical pin-tool, the flexure of this cantilever provides soft surface contact and facilitates laser-based force feedback. Design variations incorporate microfluidic channels for back loading, and multiplexed capabilities for the simultaneous deposition of multiple samples. Cantilevers that taper to a sharp point with a narrow microchannel gap will make the smallest spots. Cantilevers that have a large gap and channel acting as a liquid reservoir will make the largest spots. Some SPT designs may yield better results than others when used in conjunction with certain surfaces, samples, or sample buffers. For that reason, it is important to empirically determine the SPT that will provide the best results for each experiment.









2.10 The NanoWare™ Software

2.10.1 Limitations to the Nano eNabler

It is important to completely power down the Nano eNabler before unplugging or plugging in components. The power connector integrated into the camera is susceptible to shorting out and restarting the system if plugged/unplugged at an angle.

2.10.2 Limitations to the NanoWare™Software

Occasional glitches attributed to the Nano eNabler hardware, software, the LabView programming environment, additional software installed by the user, or the Microsoft operating system may cause the system to become unstable and freeze. This can be reduced by minimizing the installation of other third party software applications. If freezing should occur, the recommended course of action is to manually raise the Coarse Z stage (by hand) to a safe height, and then restart the system. If the problem occurs frequently, or is repeatable, please contact BioForce to file a bug report.

Creating or importing very large Array Layouts may cause a temporary slowdown or lock up while the software calculates the coordinates for each position. Wait until the software becomes responsive again before attempting further operations.

3 Nano eNabler™ Instrumentation —The Nano eNabler™

3.1 Some Features of the Nano eNabler™

3.2 Introduction to The Nano eNabler™

3.3 Operation of the Nano eNabler™

3.1 Some Features of the Nano eNabler™

3.1.1 Precision Motion Control

The Nano eNabler has three major moving components. The first is the Coarse Z stage, upon which the optical microscope and head are mounted. This allows the user to raise or lower the SPT with 45 mm of travel for coarse positioning. The second is the Fine Z stage, which is a piezo driven vertical stage that raises the printing substrates into contact with the SPT. The Fine Z stage has 100 microns of travel with a 100 nm step size. The final moving component is the XY stage assembly, which positions the printing substrates laterally with respect to the SPT. There are actually two separate piezo stages here, with one for each axis. Each axis has 50 mm of travel with 20 nm step resolution.

3.1.2 High Resolution Optical Microscope

The parfocal optical system has a 6.5:1 zoom ratio, a 0.7X to 4.5X zoom range, and total magnification of 150X to 1000X. The working distance is approximately 33 mm. The achievable field of view can vary from 0.260 mm to 1.72 mm.

http://machinevision.navitar.com/pages/product_information/hi_mag_zoom_lenses/zoom6000_overview.cfm?nav1=true

3.1.3 Laser Monitored Force Feedback

As described previously in sections 2.5.2 and 2.5.3, a laser/photodetector system is employed to monitor the contact event between an SPT and the printing surface. An optimally configured system with an SPT spring constant of approximately 1 N/m should result in sub-micronewton contact forces.

3.1.4 Environmental Control

The environmental chamber which covers the Nano eNabler instrumentation can be used to control humidity from 25-80% RH. This feature is critical for users in extreme environments such as humid summers or dry winters. Without proper environmental control, printing repeatability may suffer.

3.2 Introduction to The Nano eNabler™

3.2.1 Installation

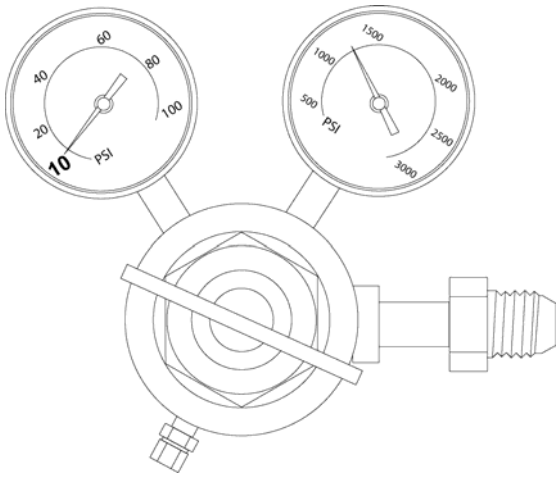
The Nano eNabler™ System is to be uncrated and professionally assembled and installed only by BioForce personnel or authorized distributors.

Vibration Isolation

The Nano eNabler™ is designed to be a robust, high resolution instrument, however the performance can be compromised by placing it on a weak or unsteady table. There are four black rubber feet affixed to the bottom of the aluminum base which help isolate the instrument from vibrations. Suggested solid surfaces upon which to place the Nano eNabler™ include a laboratory bench top, or a heavy, older-style metal or wooden desk. Unsteady surfaces to avoid include lightweight, inexpensive tables or any surface that already has a piece of equipment that may be producing mechanical vibrations. If the Nano eNabler™ cannot be set up on a solid table, at least position it toward one end to keep the weight distributed over the legs rather than in the middle of the table. Users may appreciate positioning the Nano eNabler over a knee-hole, and those users with shorter arms may also appreciate having the aluminum base positioned as close as possible to the front edge of the table or bench.

Environmental Control and Gases

Procure one 285 cu.ft. tank of an inert, dry gas such as nitrogen. Best results have been achieved with nitrogen. Do not use a flammable gas such as hydrogen or oxygen. Use research grade gas of at least 99.9995% purity with moisture <0.5 PPM for best results. Secure the gas tank to a wall or other solid surface using an anchor strap, chain, or other method sanctioned by your institution. Attach a high quality regulator valve with the correct fittings according to the manufacturer's instructions. Thread a barbed brass nipple onto the regulator. Connect a length of Tygon™ tubing from the regulator to the connector labeled "Dry Gas In" on the back of the Nano eNabler™ Controller. Open the main valve on the gas tank and adjust the regulator valve to 5-10 psi (pounds/square inch). Five psi is generally sufficient for a single Nano eNabler™, however you should use at least 10 psi to supply more than one Nano eNabler™.



Place the Environmental Control Chamber over the aluminum base and arm of the assembled Nano eNabler™. Unscrew the cup on the humidification device attached to the outside of the environmental chamber and add ddiH_2O to the fill line.

Match up and connect the labeled end of the quick disconnect tubing with the corresponding connectors on the back of the Nano eNabler™ Controller and the back of the Environmental Control Chamber.

Cabling

The cabling consists of Tygon quick disconnect tubing and 3-pin humidifier power cable which connects to the back of the Nano eNabler. The Tygon tubing would go to the back of the Environmental chamber and the power cable would connect to the bottom of the humidifier.

Computer Setup and Software

The Nano eNabler™ System ships pre-loaded with the software necessary to operate. In the event that the system requires reinstallation, low-level Acronis True Image software has been installed on the Nano eNabler™ that will allow the system to be fully restored to its original shipping state by pressing the F11 key at bootup, and selecting the “Restore Image” option. For additional assistance, please contact support.

3.2.2 Environmental Control for Instrument

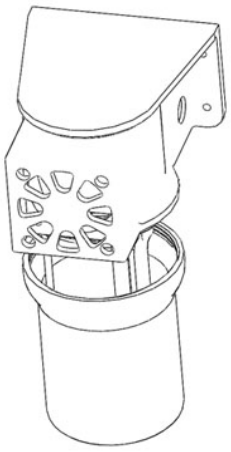
Environmental control is critical for successful and reproducible printing. Ambient room humidity has a significant effect on spot size and printing success, and must be eliminated as a factor. We have designed an efficient and stable environmental control system that is composed of an enclosure, a temperature and humidity sensor, and a computer controlled feedback loop.

Environmental Enclosure

General environmental control for the Nano eNabler™ is provided by the custom enclosure. The large door on the front panel allows access for initial setup of samples, chips, and Surface Patterning Tools. Open the door by pulling the handle toward you, rather than lifting straight up. After closing the door, desired humidity can be set within the software.

Temperature and Humidity Sensor

A temperature and humidity sensor is mounted on the back right side of the aluminum arm. The humidity sensor has a reported accuracy of +/- 2% RH from 0-55% RH. Output voltages from the sensor are converted into relative humidity values by the Nano Ware™ software. See Section 3.3.2 for a more detailed description of the environmental controls within the NanoWare™ software.



Flow Control for Humid Air

Humid air for the general environmental control is generated by pulling air from outside the enclosure through a custom humidification device into the enclosure. Relative humidity levels inside the chamber of 80% or greater can be attained using this process. The NanoWare™ software monitors the humidity within the enclosure and automatically starts or stops the humidification device responsible for raising the humidity. Check the ddiH_2O humidification device regularly to ensure that the water is not discolored or cloudy, as this is an indication of microbial growth.

Flow Control for Dry Air

Dry air for the general environmental control system is provided by the tank of dry, inert gas that was connected to the Nano eNabler™ system in Section 3.2.1. The

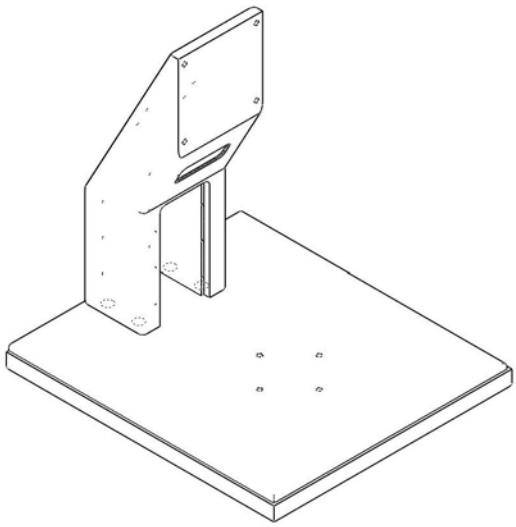
NanoWare™ software monitors the humidity within the enclosure and automatically opens or closes a solenoid that dispenses dry gas into the chamber.

3.2.3 Motion Control Systems

The Nano eNabler™ has been designed from the ground up for precision, stability, and throughput. Each component represents the best available compromise between precision, speed, and maximum travel.

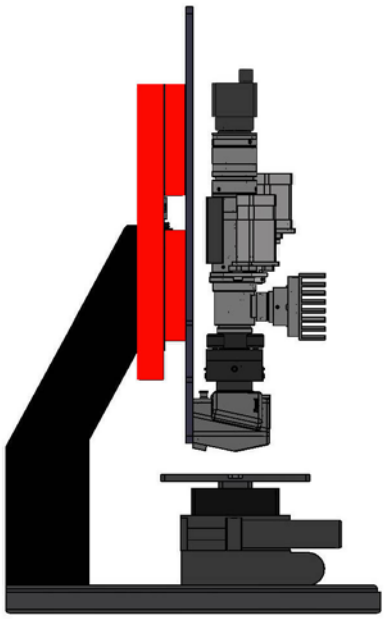
Aluminum Base and Arm

A custom aluminum base and arm weighing approximately 14kg (31lbs.) provides a stable platform for the entire instrument.



Coarse Z Stage

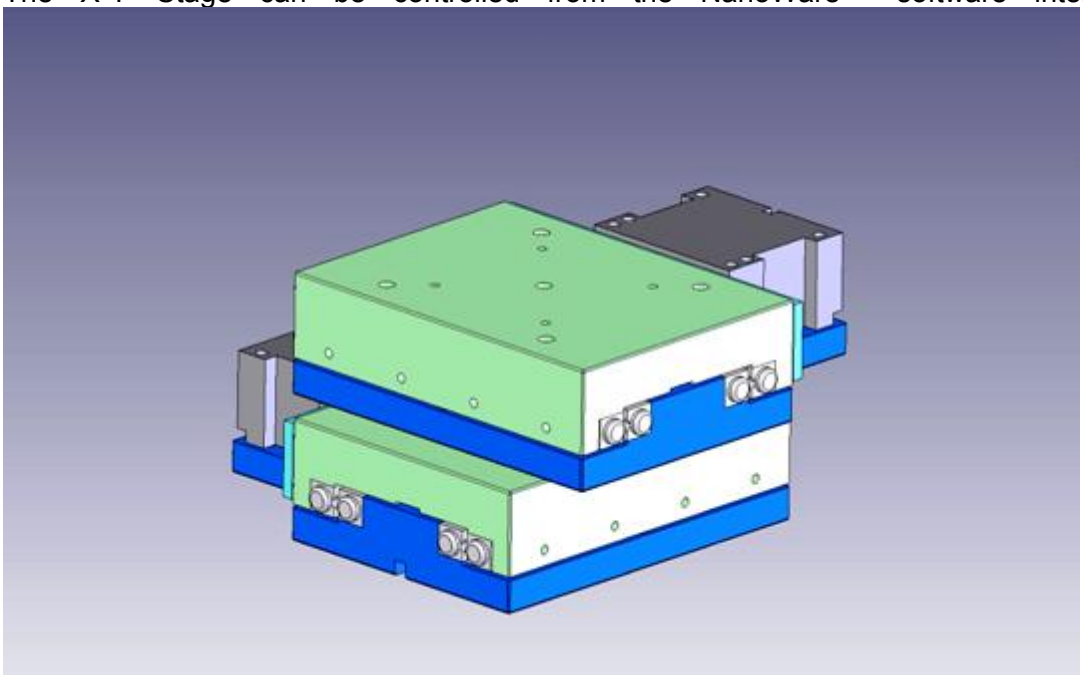
Intended to raise and lower the entire optical microscope and Head assembly, this high precision stage is “coarse” by name only. The primary considerations for the Coarse Z Stage are speed and maximum travel. This unit offers 45 mm of travel. Despite being referred to as coarse, it is equipped with a 434 nm resolution rotary encoder that allows sub-micron repeatability. The Coarse Z Stage is controlled via the NanoWare™ software interface



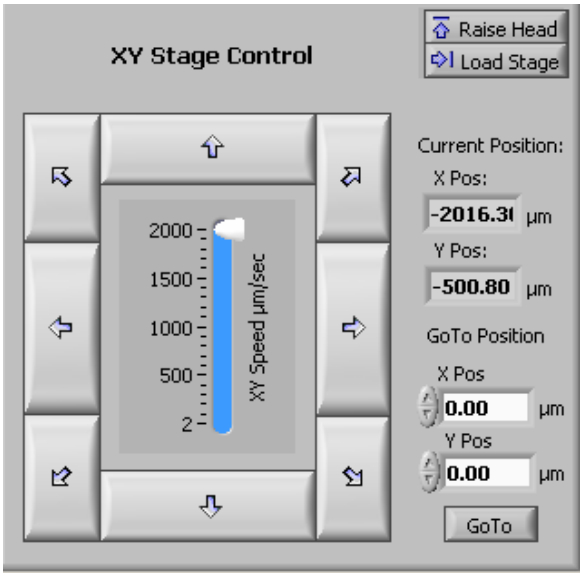
High Precision X-Y Stage

The high precision X-Y sample stage offers a large 50 mm x 50 mm working area for maximum flexibility, and speeds up to 2.23 mm/second to get the job done quickly. The linear encoders have 20 nm resolution.

The X-Y Stage can be controlled from the NanoWare™ software interface.



The software interface offers user-configurable sets of buttons for X-Y navigation. The ring of buttons is intended for high precision movements with adjustable speed using the scroll wheel when the curser is over the vertical slider located in the center of the buttons. More details on X-Y navigation using the software interface can be found in Sections 2.4 and 3.3.2.



Fine Z Stage

The Fine Z Stage is the component that is actually responsible for bringing the surface into contact with the SPT from underneath.

NOTE: The SPT is stationary and does not move down to touch the surface.

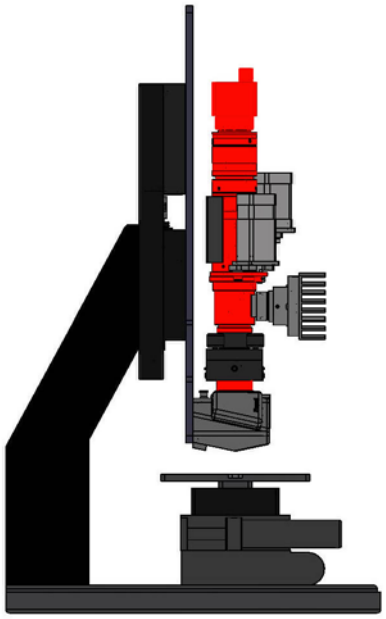
This is a key concept to understand before using the Nano eNabler™. The Fine Z Stage has 100 μm of total travel, 1nm resolution and full range repeatability of +/- 20 nm.

Sample Platform

The sample platform is an aluminum plate that has been machined to very tight tolerances, held down by magnets, in order to provide a flat surface to secure the loading and deposition substrates. The recommended method for attachment of glass slides or silicon chips to the sample platform is with BioForce Sindex™ adhesive pads or 3M Scotch Removable Double Stick Tape. This tape will hold samples securely without leaving gummy residue.

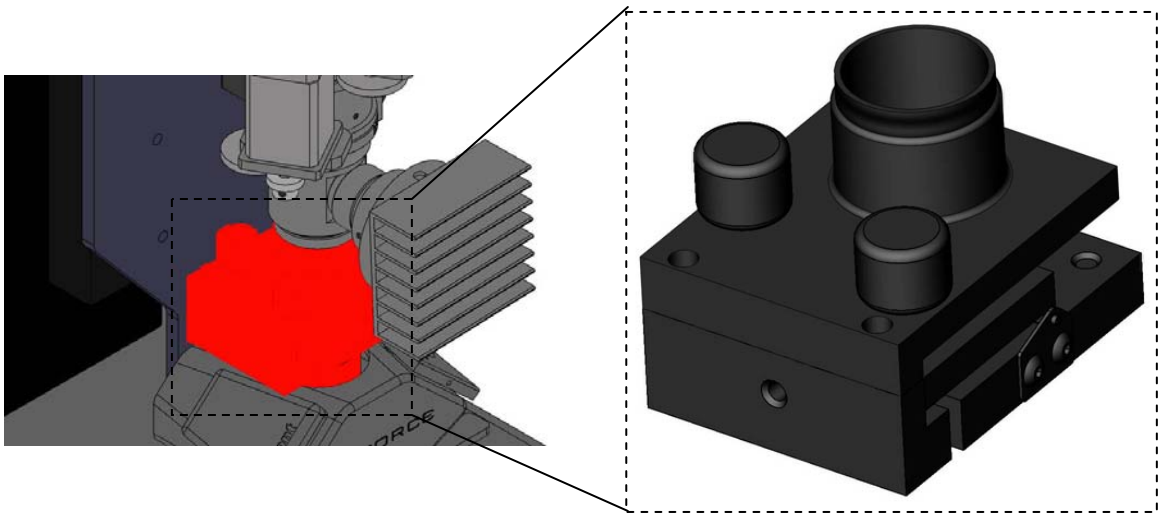
3.2.4 Optical Microscope

The powerful optical microscope system integrated into the Nano eNabler™ allows the user to observe the deposition process, even with sub-micron spots. The motorized zoom permits high magnification viewing for monitoring depositions as well as wide angle viewing of the SPT or surfaces.



X-Y Control

The optical microscope objective is anchored by a pivot point at the bottom of the stack, and X-Y translation of the microscope is accomplished with the two thumbscrews near the bottom of the microscope. Each thumbscrew pushes against the spring-loaded inner collar and pivots the microscope's objective around the anchor point. Use these thumbscrews to position the microscope such that the SPT is centered in the image from the camera.



Zoom Control

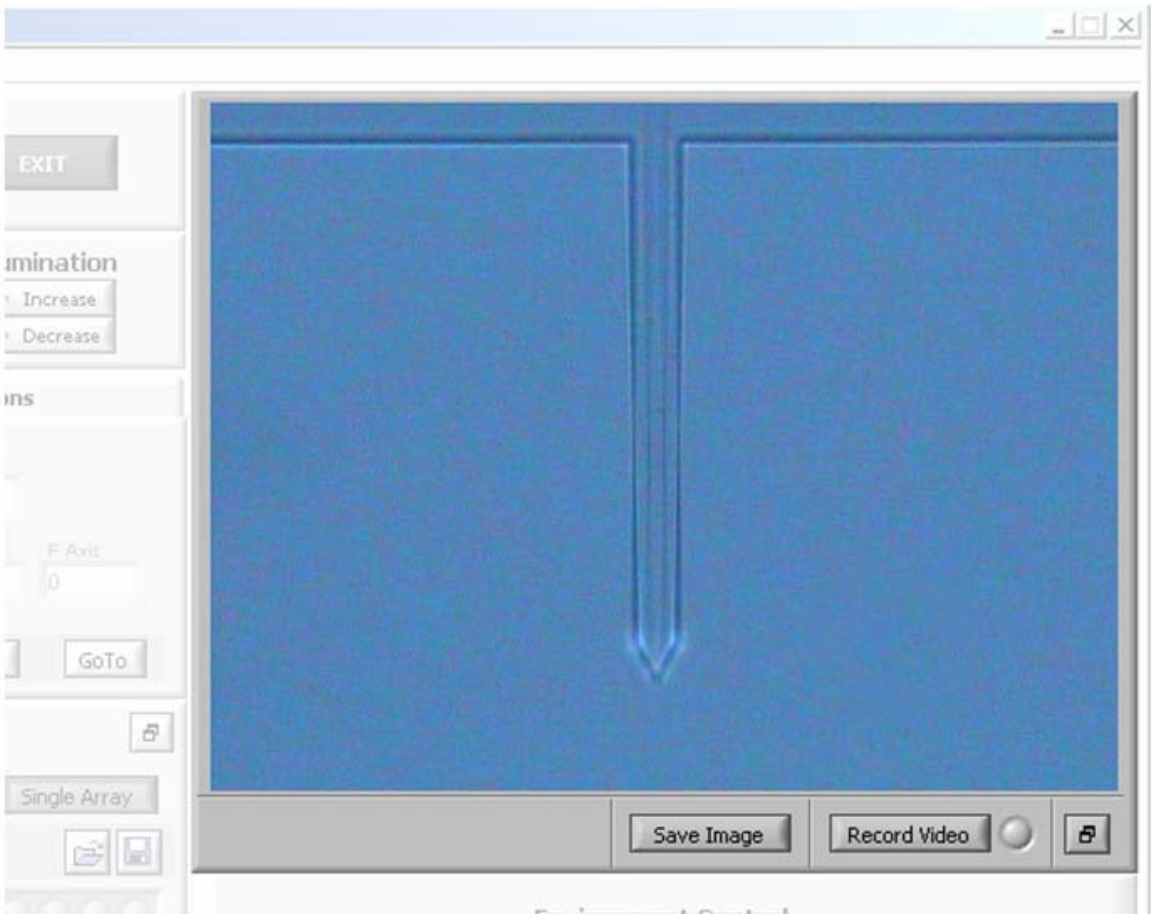
The optical microscope has a 6.5:1 motorized zoom that is controlled from the NanoWare™ software interface. There are two simple buttons designated as Zoom In and Zoom Out that allow adjustments in zoom. See Section 4.2.3 for more information.

Focus Control

Focus controls are motorized and controlled through the NanoWare™ software. There are two fast focus up/down buttons and two slow focus up/down buttons. The fast focus buttons are convenient for large changes in focal plane, such as when you are switching between the SPT and the surface. The slow focus buttons are convenient for making precise, subtle changes in focus. There is also another set of buttons that allows the user to set particular focal planes, such as the SPT or chip surface, and go directly to those preset focal planes. Due to some inherent mechanical issues with this focus system, users may notice minor backlash when manually focusing or going to a preset focus position. More details concerning the focus control software features can be found in Section 4.2.3.

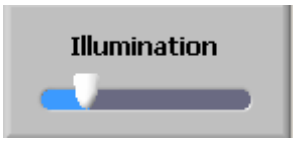
Imaging Camera

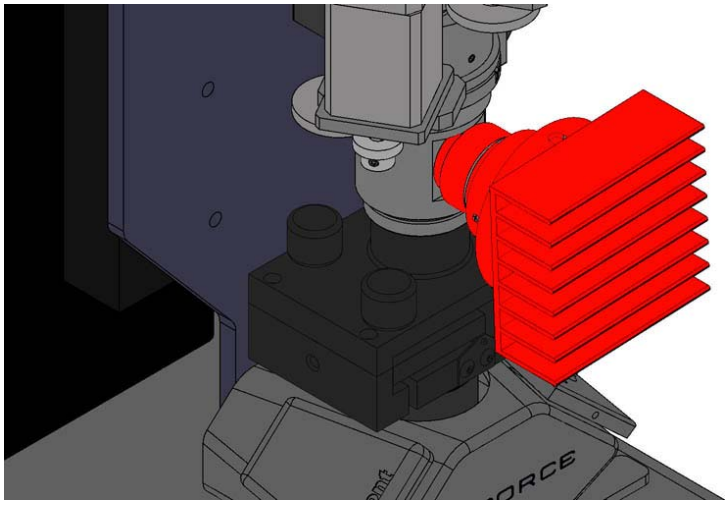
The imaging camera is a 1/3" color CCD with 640 x 480 pixel resolution. Output is standard NTSC through a coax cable to the video capture device in the controller. It is recommended that the camera use factory defaults, although many settings are adjustable via the buttons located on the top of the camera using on screen display. Video and images may be saved using the Save Image and Record Video options below the display. A full sized 640x480 sized video window may be displayed by clicking the 🗗 icon.



LED Light Source

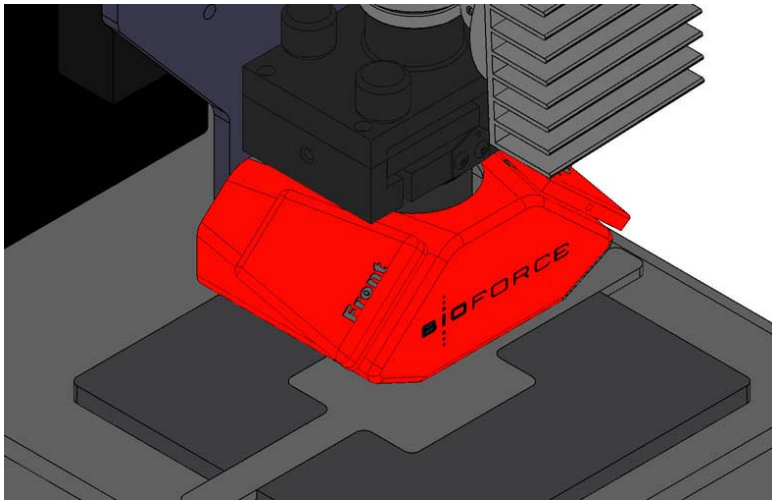
An adjustable LED light source provides illumination for the optical microscope within the NanoWare™ software via the Increase and Decrease Illumination slide bar.





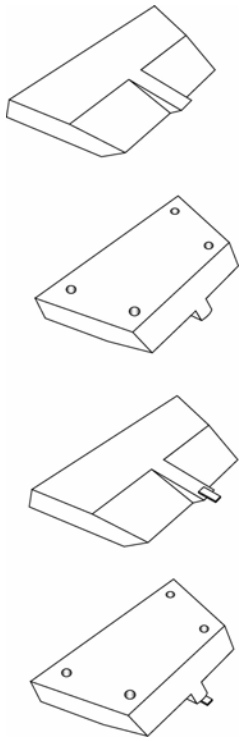
3.2.5 Multi-Component Head

The Multi-Component Head contains the Surface Patterning Tool holder, the laser/photodetector force feedback system.



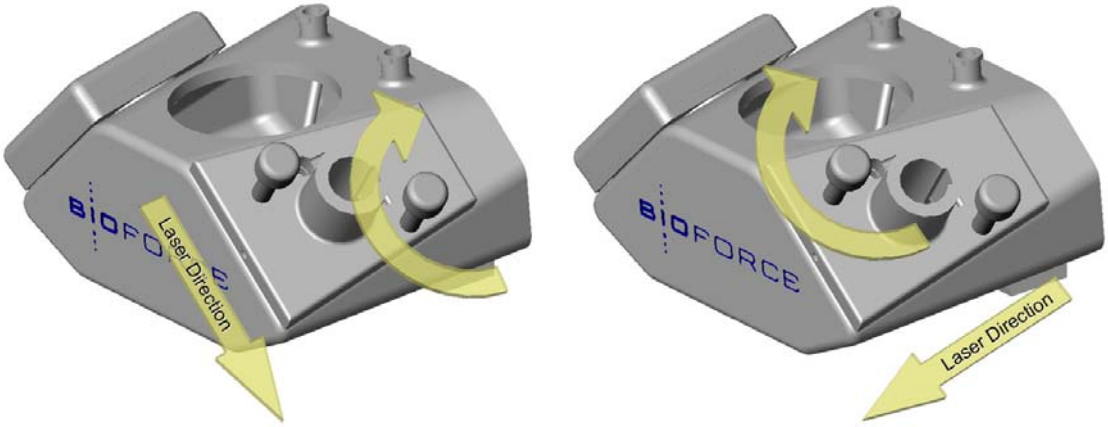
3.2.6 Surface Patterning Tool Holder

The SPT Holder is aligned and attached to the Head via small, powerful magnets. It is easily separated from the Head by gently applying pressure to the sides of the tool holder with your thumbs, and pulling toward you. This should only be done with the Coarse Z Stage in its highest "loading" position. Surface Patterning Tools are mounted on the tool holder using BioForce SPT adhesive pads, or 3M Scotch Permanent Double Stick Tape. Position the SPT such that the front edge hangs over the edge of the tool holder by approximately 1-2mm.



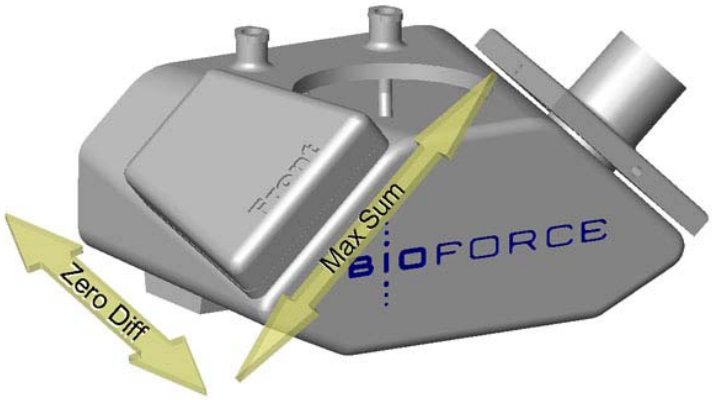
3.2.7 Laser

The focused, variable-intensity 0.9 mW, 635 nm red diode laser is mounted on the right side of the Head, shining down onto the cantilever and reflecting back up to the photodetector on the left side. Two thumbscrews on the adjustable laser mount allow proper positioning of the laser onto the cantilever.

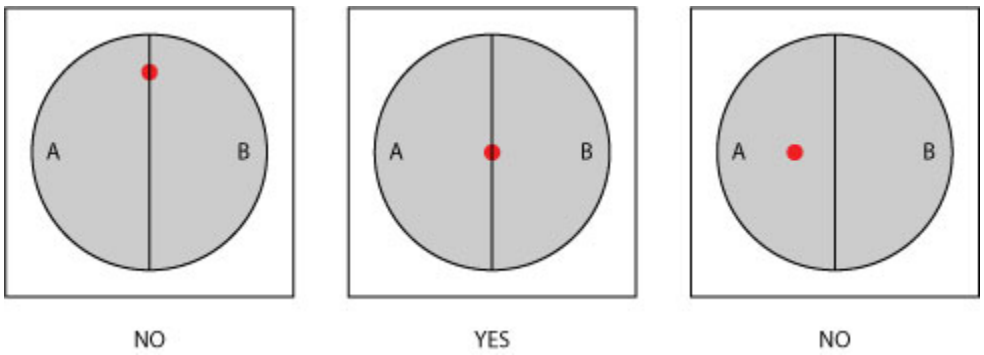


3.2.8 Photodetector

The split photodetector is held against the left face of the Multi-Component Head magnetically. This mounting arrangement allows for maximal flexibility in positioning. Simply slide magnet-mounted photodetector back and forth by grasping the photodetector puck. Since the surface-induced deflection of the cantilever moves the reflected laser beam from front to back along the left side, it is important to keep the front of the puck parallel to the front of the head. This ensures that the beam deflection will be perpendicular to the split between the two halves of the photodetector, which maximizes the sensitivity of the unit.



As depicted in the illustration below, the photodetector puck should be positioned such that the laser beam strikes the center of the photodetector. The first example is incorrect because the Sum is likely not maximized, and the path of the beam will move off of the photodetector as the SPT deflects. The third example is incorrect because although the Sum may be high, the laser spot is not evenly divided between the A and B halves of the photodetector. The center example depicts a properly positioned photodetector, with the laser centered vertically for maximum Sum and laterally for a zeroed Difference.



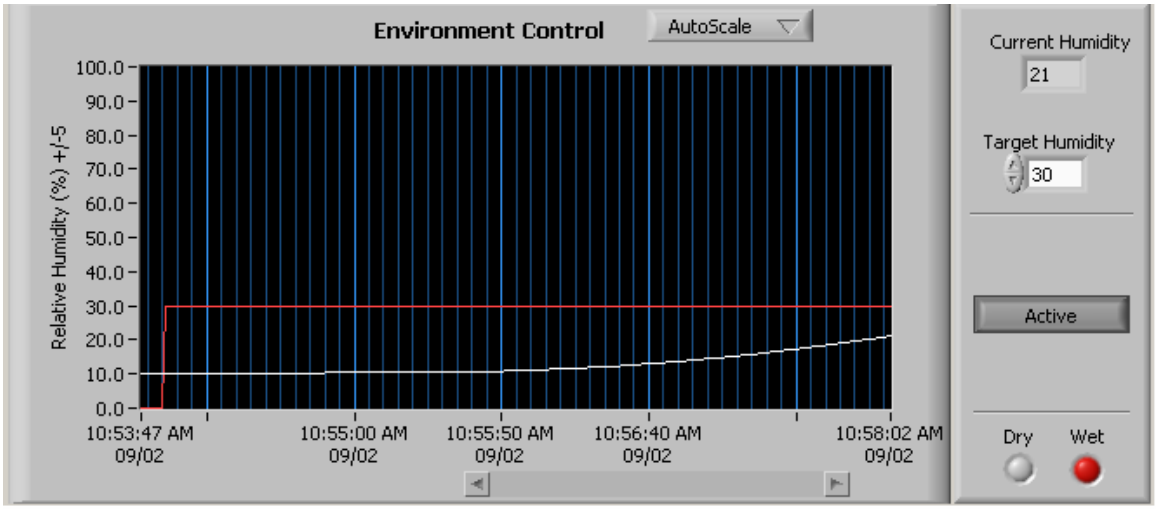
3.3 Operation of the Nano eNabler™

3.3.1 Startup

Sequence of Events

- Start up the computer
- Open the valve on the dry, inert gas tank
- Open the NanoWare™ Software
- Enter in the desired humidity
- Raise Coarse Z Stage to Load position
- Mount SPT onto holder and load with solution(s)
- Press holder onto Multi-Component Head
- Load surfaces to be patterned onto the sample platform
- Close the environmental chamber door

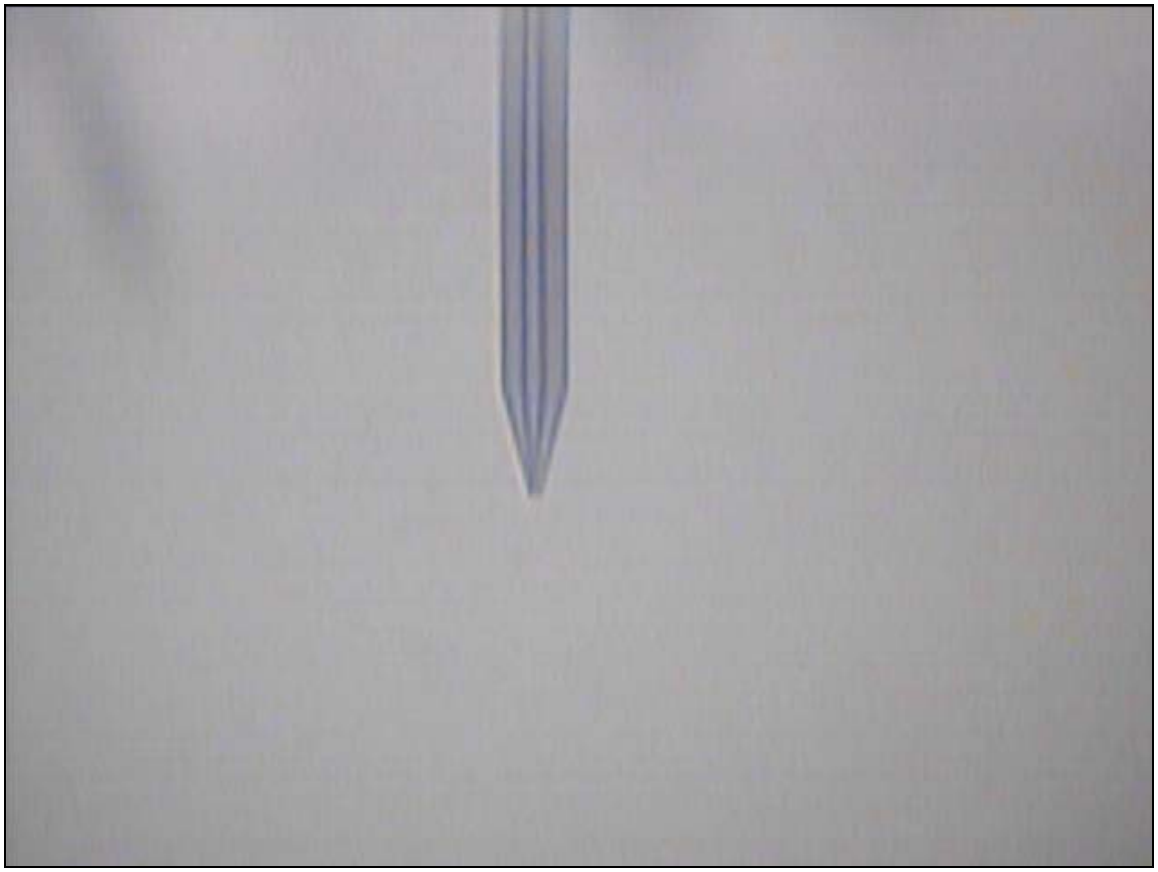
3.3.2 Environmental Control



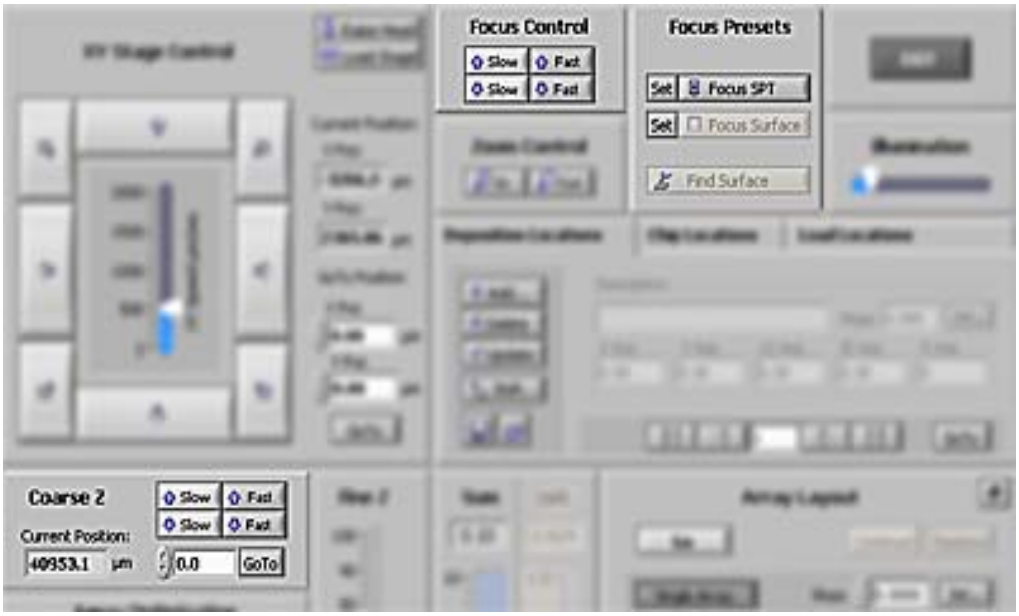
Close the front door after the SPT and surfaces have all been placed inside the environmental enclosure. In the NanoWare™ software, enter a desired humidity level. Monitor the changes in relative humidity on the scrolling chart. The values along of the vertical axis can be customized by double-clicking the top or bottom number and typing in a new range.

3.3.3 Optics and Camera

Zoom out to the widest field of view and use the X-Y adjustment thumbscrews to locate the SPT. Position the end of the SPT in the center of the video image. Slowly focus up or down to fine tune the focus on the cantilever. Zoom all the way in to the highest magnification and make any necessary adjustments to the focus and X-Y centering of the optics.



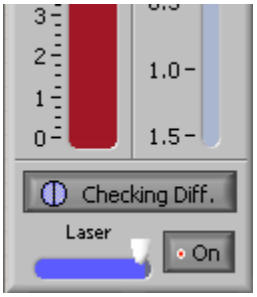
When the SPT is in focus, press the Set button below the Focus Tool button to save the position of that focal plane. The optical system will focus down to its limit and then come back to the desired focal plane of the SPT to remove any mechanical backlash in its gears.



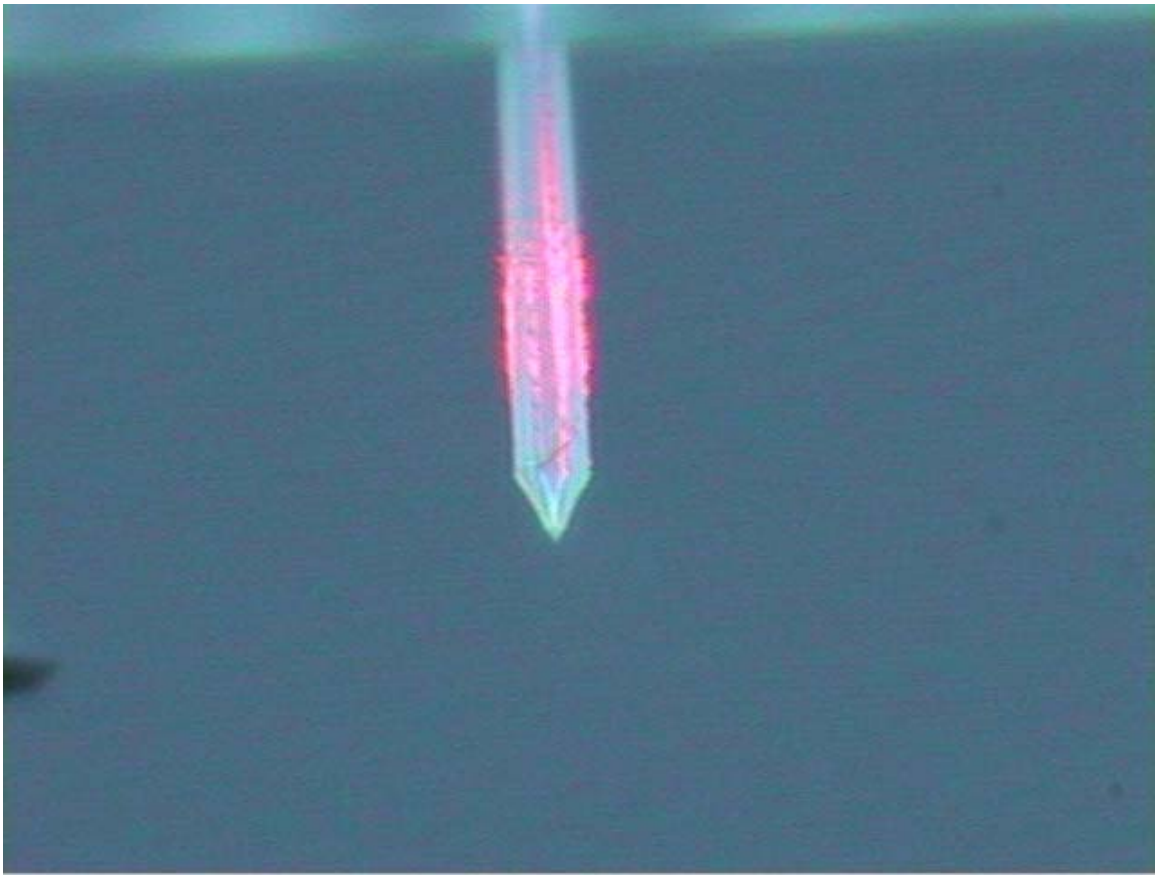
Due to the backlash in the focusing mechanism, you may need to fine tune the focus and set it again. To minimize the slack or backlash in the optics system, it is recommended, but optional, that the last focus movement is upward.

Use the Coarse Z Stage Down Fast button to bring the Multi-Component Head and optics down to within 3 mm of the surface you would like to touch. Focus Down Fast until the surface is in focus. Manipulate the X-Y Stage using software to find the area of interest. Use the slow focus controls to bring the surface into sharp focus and then press Set for Focus Surface. With the focal planes of the SPT and surface now defined, the Find Surface feature will be able to automatically engage the surface once the laser and photodetector is set.

3.3.4 Laser and Photodetector

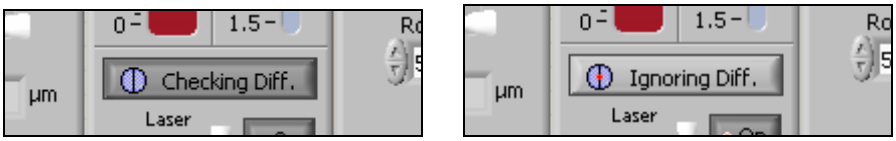


When starting the software the laser will default to its on state. To turn the laser on or off, toggle the On/Off button near the laser intensity slider below the sum and diff. Increase the laser slider intensity to its maximum by moving it to the far right. Rough initial positioning of the laser is often most easily accomplished by the naked eye rather than using the optical microscope. Move the thumbscrews back and forth until you find the laser beam striking a solid surface such as the silicon cantilever substrate or the SPT holder. Then use the thumbscrews to move that beam into the field of view of the optical microscope at its lowest magnification (zoom out completely). Adjust the light source illumination as necessary to avoid overpowering the CCD camera, causing the image to become washed out. Position the laser beam on the cantilever using the two thumbscrews. The system will be most sensitive with the laser beam focused 2/3 from the base of the cantilever.

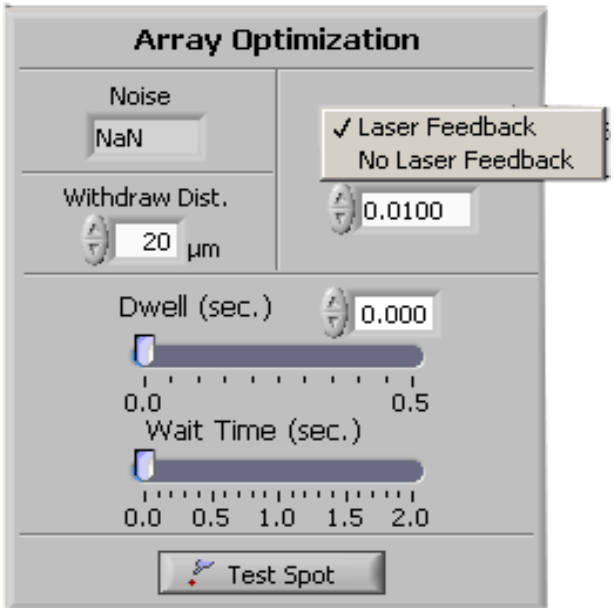


Next, slide the photodetector back and forth and up and down by hand to maximize the Sum meter as high as possible without saturating it. The optimal Sum voltage is 8-9 volts on the 10 volt meter.. You may need to go back and reposition the laser or adjust the laser intensity for the best Sum. Next, adjust the photodetector to bring the value of the Difference as close to zero as possible. This value is displayed graphically to the right of the Sum meter and numerically above that. During the patterning process, the

Difference may drift due to heat, humidity, air flow, added mass, surface reflection, or any number of variables. The surface detection system loses sensitivity as the Difference value drifts away from zero. Therefore, a Difference-checking feature is integrated into the software that warns the user to fine tune the position of the photodetector to bring the Difference back to zero whenever it gets outside of the normal operating range. To turn off that feature, press the button labeled “Checking Diff” and it will change to “Ignoring Diff.” This is not recommended for new users. If the Sum drops below 2, a warning will be displayed to caution the user that the laser or photodetector may be positioned improperly. Another potential reason for the Sum dropping below 2 could be that the SPT has crashed into a surface without triggering the force feedback system.



In the Array Optimization section is a pull down menu with the options Laser Feedback, and No Laser Feedback. These options allow the user control over the contact force.

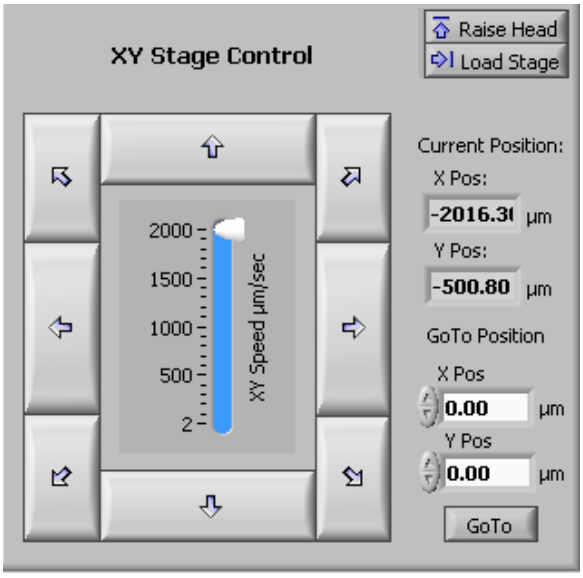


The Laser Feedback mode allows the user to directly input a desired contact force. No Laser Feedback mode may be used when the tool is at a known distance from the surface and/or printing requires more speed.

3.3.5 X-Y Stage Movement and Control

Within the software, manual movements can be made using the rings of directional control buttons. Their speed is set using the vertical slider or scrolling the track wheel in the center of the ring (set in µm/sec). Additionally, if you know the coordinates of the position you would like to move to, the “GoTo” position feature can be useful. It is

possible to record separate preset locations for loading or depositing materials, and that feature is discussed in detail in Section 4.2.4. Moves using these buttons are relative to the fixed tool position, so when watching the stage move (not in the video) directions may seem inverted.

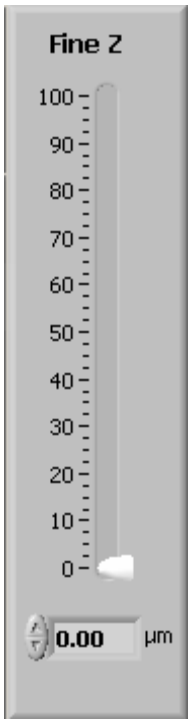


3.3.6 Coarse Z- Stage Movement

The software interface uses two sets of buttons to manually control the Coarse Z movement. Each up button is labeled Fast or Slow with its corresponding down button beneath. Below those four buttons is a grayed-out box that displays the current Coarse Z position. An up move is negative and down is positive negative in this display. Beneath that display is a box for entering a new Z position and a “GoTo” button to make a move.

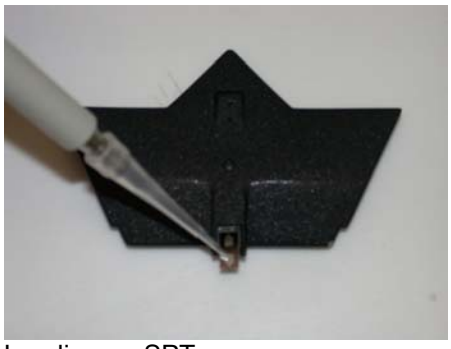
3.3.7 Fine Z- Stage Movement and Control

The Fine Z Stage has 100 μm of vertical travel and it is controlled from the NanoWare™ software interface. Immediately to the left of the Sum/Difference meter is a box labeled Fine Z, with a vertical slider inside. This slider is used to manually raise and lower the Fine Z Stage. The scale to the left of the slider indicates the position and amount of vertical travel of the stage in microns.



3.3.8 Sample Loading

For creating arrays of biomolecules, the preferred method of loading the SPT will depend upon the ultimate density of the array. Loading the SPT involves pipetting a small volume (generally less than 0.5 μl) of sample into one of the wells etched into the top surface of the silicon SPT substrate. Sample will fill the well and flood the channel that runs down the length of the cantilever. SPTs should be pretreated with UV and ozone in a BioForce TipCleaner™ for at least 30 minutes prior to either back-loading or front-loading.


Loading an SPT

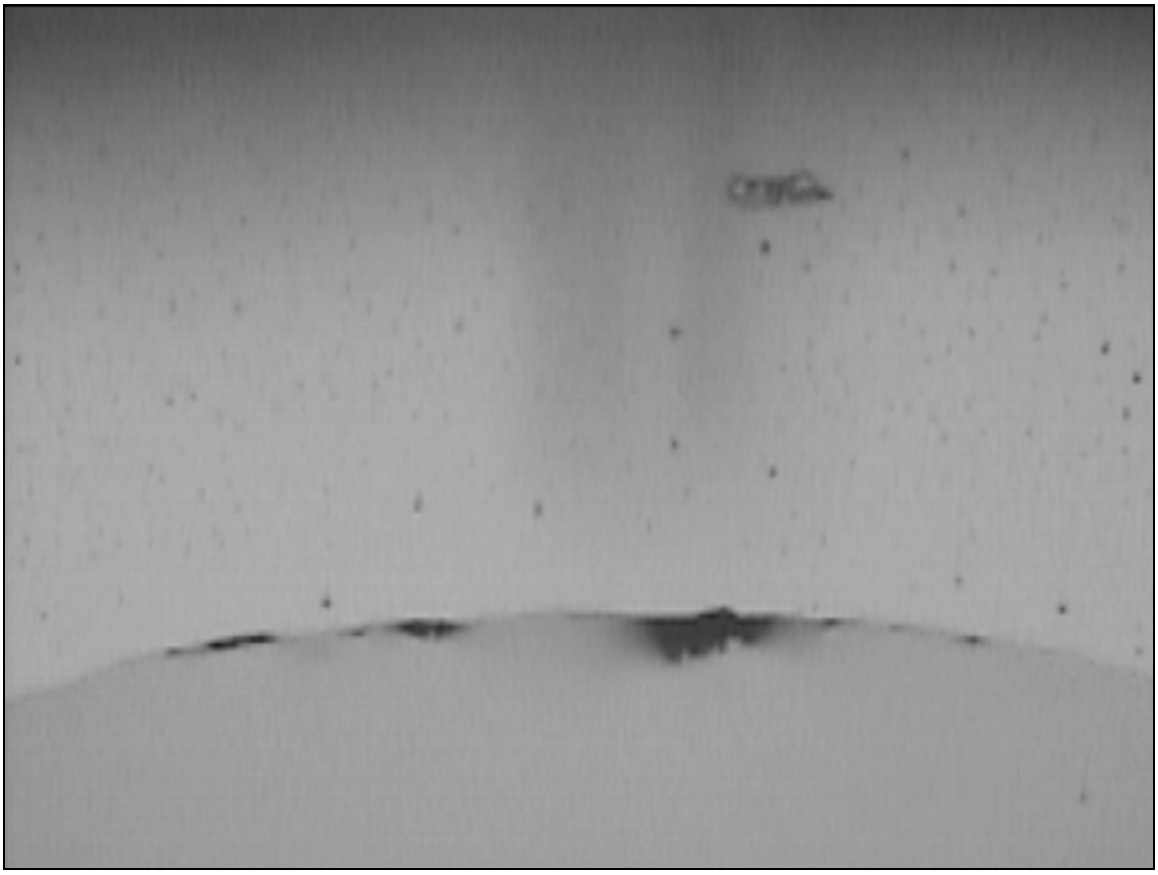
To load an SPT, the first step is to mount it on the SPT Holder using a BioForce SPT adhesive pad or a piece of 3M Permanent Double-sided Stick Tape. Once the SPT is secure, use a 10 μl pipette to dispense approximately 0.5 μl straight down into the reservoir. The SPT and SPT Holder may be placed on the sample platform to allow high magnification observation of the loading progress. As liquid fills the channel and flows toward the end of the cantilever, the channel will begin to disappear. If the channel is still easily visible, then it may not be fully loaded. If dispensing additional liquid into the reservoir or placing the SPT in a humid environment are not sufficient to fill the channel, it may be an indication that there is a blockage. If that should occur, wash the SPT with a stream of ddiH_2O and treat it with UV/Ozone in the BioForce TipCleaner™ for 30 minutes before attempting again.

Loading SPTs with multiple cantilevers and reservoirs for multiplexed printing requires a slightly different approach. Mount the SPT on the SPT Holder as above. Aspirate 0.5 μl of solution into the pipette tip, then remove the pipette tip from the pipette. Place a finger over the wide end of the pipette tip such that applying gentle pressure would cause a small volume of the liquid to be dispensed. Next, position the dispensing end of the tip vertically over one of the SPT reservoirs. Touch the pipette tip to the silicon post in the center of the reservoir. If no liquid wicks out into the reservoir, apply a slight pressure with your finger to actively dispense a few hundred nanoliters. This approach generally offers greater volume control than most standard 10 μl pipettes. If an excessive amount of liquid were dispensed into the reservoir of a multiple cantilever SPT, there is a high chance of cross contamination between wells and channels.

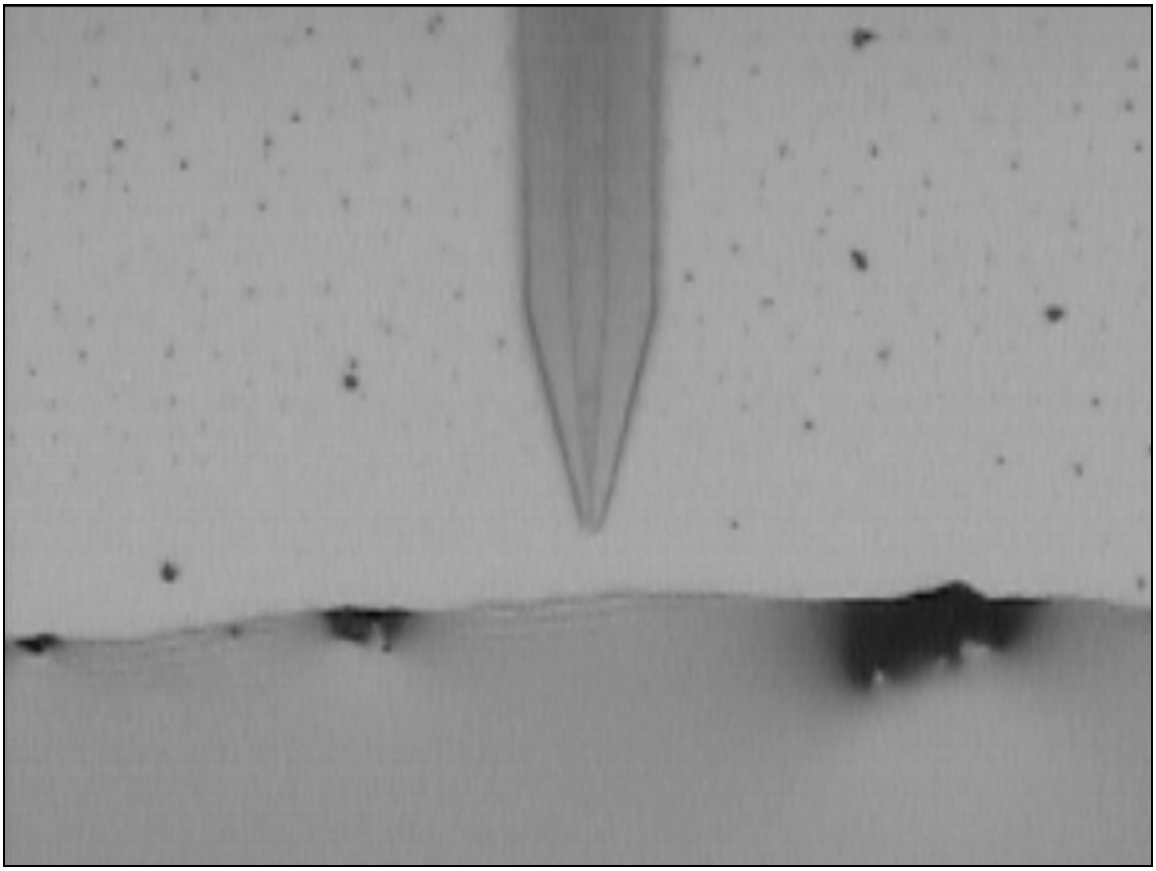
An alternate method of loading may be accomplished using either a compatible loading chip containing a matching set of wells and loading channels, or by immersing a cantilever into a droplet of liquid. For patterning a small number of compounds it would be sufficient to place a few hundred nanoliters to a microliter of each material on a glass coverslip with a pipette tip. Coverslips can be pre-treated with Sigmacote™ to decrease spreading of the droplets. For printing a large number of biomolecules it may be helpful to first create a loading slide using a standard microarrayer. Use either a pin tool or ink jet microarrayer to spot down each compound on a clean glass slide. Since binding is not desired on the loading slide, avoid slides that have been treated with surface chemistries to enhance binding. Do not wash the slide after printing. Each microarray spot will represent a loading reservoir for a virtually unlimited number of spots printed with the Nano eNabler. Loading slides may be stored for extended periods at +4°C.

Mount the loading slide, coverslip, or Sindex™ chip onto the Nano eNabler™ sample platform using BioForce Sindex™ adhesive pads, or 3M Permanent Double-sided Stick Tape.

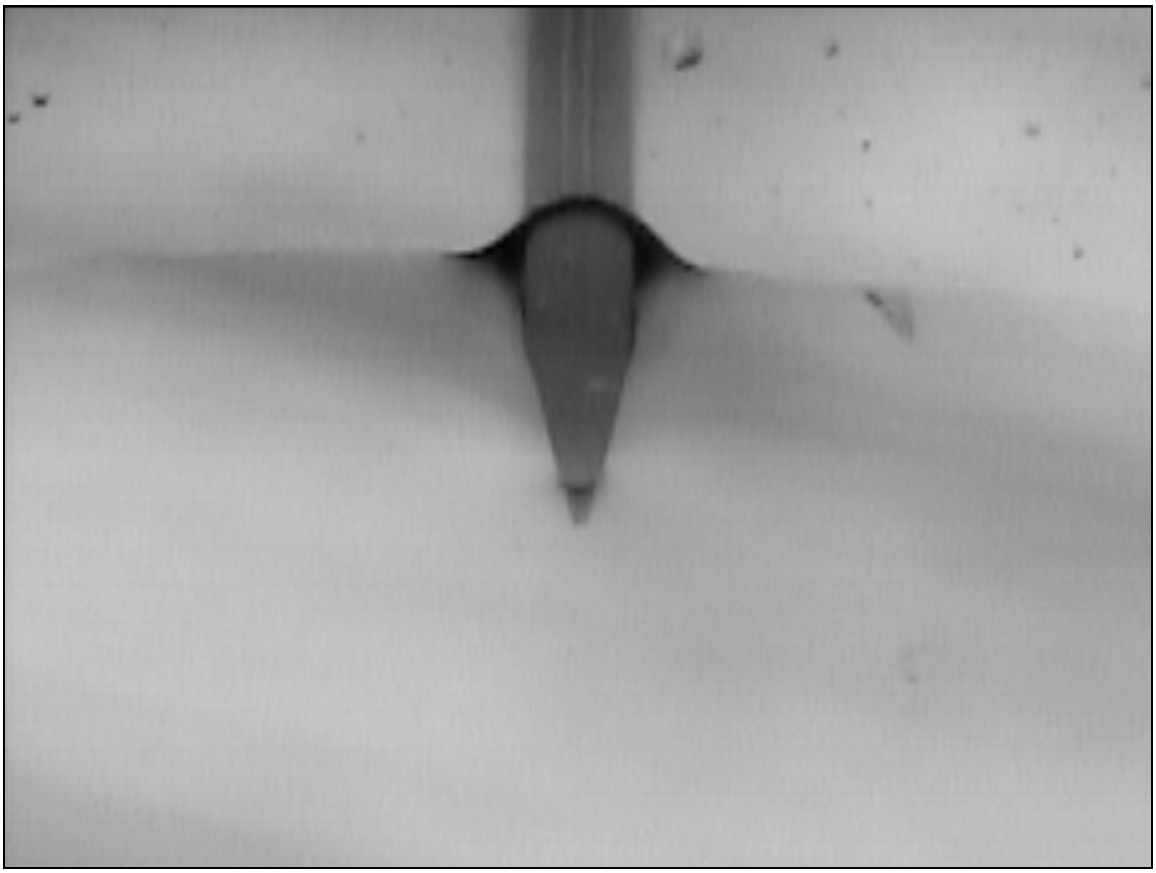
If biomolecules were prepared using BioForce Spotting Buffer, best results are achieved after allowing evaporation of water from the loading spots. This effectively concentrates the biomolecules by up to 10x. Position the SPT above the loading spot and focus down to the surface adjacent to the top edge of the spot. Set this as the Surface Focus.



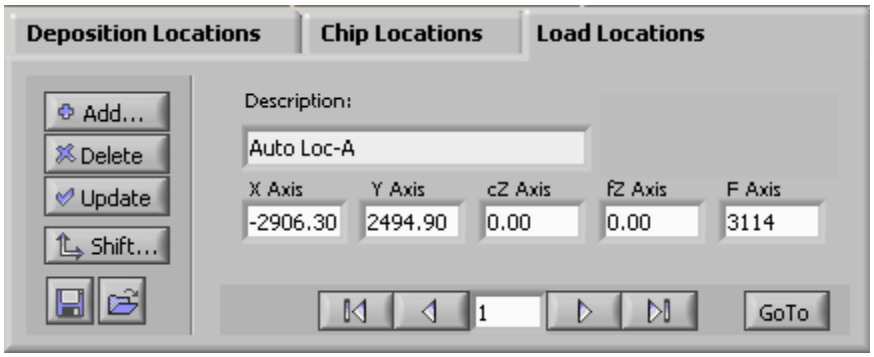
Run the Find Surface command to bring the SPT and loading surface into contact. That contact point should be on the surface next to the loading spot, and not directly in it.



Use the Fine Z Stage slider to gently bring the surface up to the plane of the cantilever. Next, use the fine X-Y controls to carefully submerge the very end of the SPT in the liquid. Immersion of the last ~50 μm of the cantilever is generally sufficient. Avoid overloading the SPT with sample. Overloaded SPTs can be difficult to control and lead to poor spotting reproducibility.



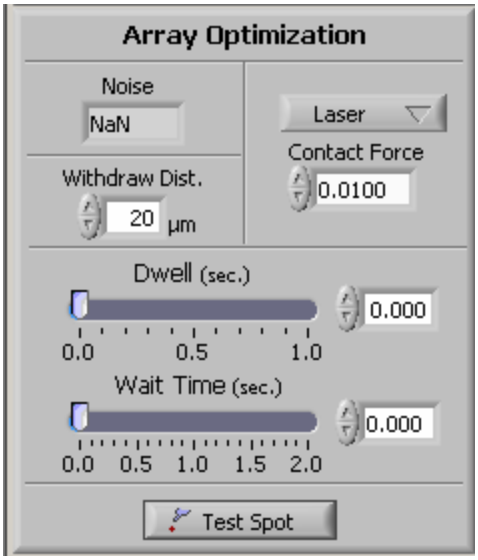
Beware capillary forces that may wick materials between the cantilever and the surface, leading to excessive loading. Set this as a pre-set load location by clicking the Add button under the Load Locations Tab and entering a description. Return to the deposition surface either manually or by using pre-set deposition locations. Pre-set locations are covered in greater detail in Section 4.2.4.



3.3.9 Sample Deposition

Set the desired Withdraw Distance based on the viscosity of the liquid to be arrayed and the spring constant of the SPT. This is the distance which the Fine Z stage will pull back following each deposition or find surface event. Stiffer cantilevers can use smaller Withdraw Distances, while weaker cantilevers need a greater Withdraw Distance to be able to break free from the surface. Shorter distances result in faster engage times for

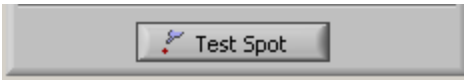
each spot. Start with 30 μm and then fine tune from there. Withdraw Distance selection is covered in greater detail in Section 4.2.5.



Engage the deposition surface using the Find Surface button (See Section 4.2.3).

Zoom in and use the focus presets to focus on either the surface in this withdrawn state or the SPT. Set the desired Dwell Time using either the slider or by typing a number directly into the box above the slider.

Press Test Spot to make a deposition.



The surface will rise up until a contact event is registered. That time point is considered Time 0.0 on the Dwell Time slider. After the Fine Z stage withdraws, the X-Y Stage will move over 10 μm in both X and Y to allow observation of the test spot. Start with small Dwell Times, such as 0.0 or 0.2 sec, and increase as necessary to produce visible spots. Adjust the humidity as necessary.

A certain amount of experimentation and optimization of these parameters is required for best results.

3.3.10 SPT Washing

Surface Patterning Tools may be washed and reused to print the next solution. Washing can either take place while mounted in the Nano eNabler, or off-line after being removed from the SPT Holder. Off-line washing should be conducted by following these steps:

Safety Precautions:

1. Follow basic lab safety guidelines.
2. Use Fume Hood while working with Acetone, Ammonium Hydroxide, Hydrogen Peroxide.
3. Wear chemical gloves when working with mixture of Ammonium Hydroxide and Hydrogen Peroxide.

Wash Process:

1. Rinse the SPTs with running ddiH_2O water for 1-2minutes.

2. Soak the SPTs in fresh ddiH_2O water for at least half an hour for three changes.

3. Soak the SPTs in 99.99% HPLC grade Acetone for 15minutes.

4. Soak the SPTs in 99.99% HPLC grade Ethanol for 15 minutes. Skip to step 7 if the SPTs cantilevers and channels are free of unwanted particles under microscope.

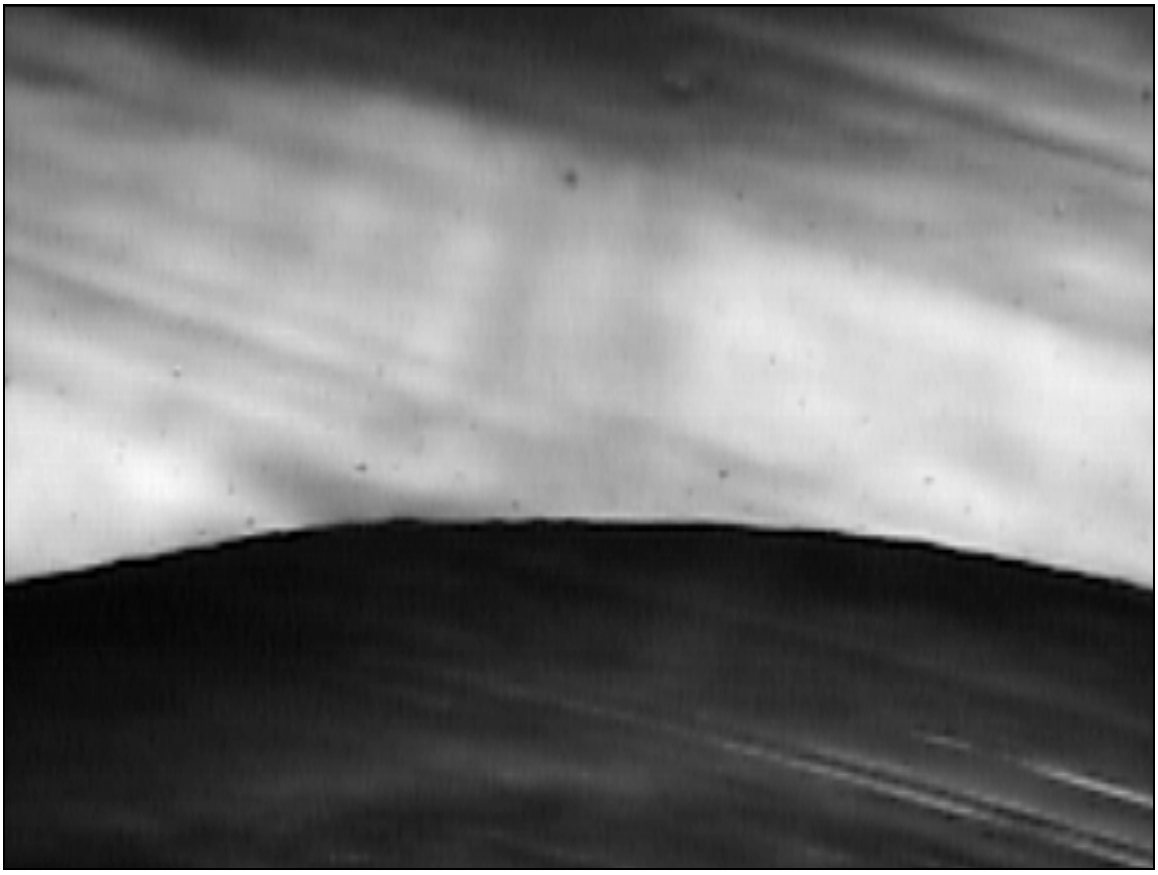
5. Soak the SPTs in a fresh 1:3 mixture of Ammonium Hydroxide (NH_4OH) and Hydrogen peroxide (H_2O_2) for 5-10 minutes. The bubbles will help to remove stuck tiny particles.

6. Rinse the SPTs with running ddiH_2O water followed by 99.99% HPLC grade ethanol.

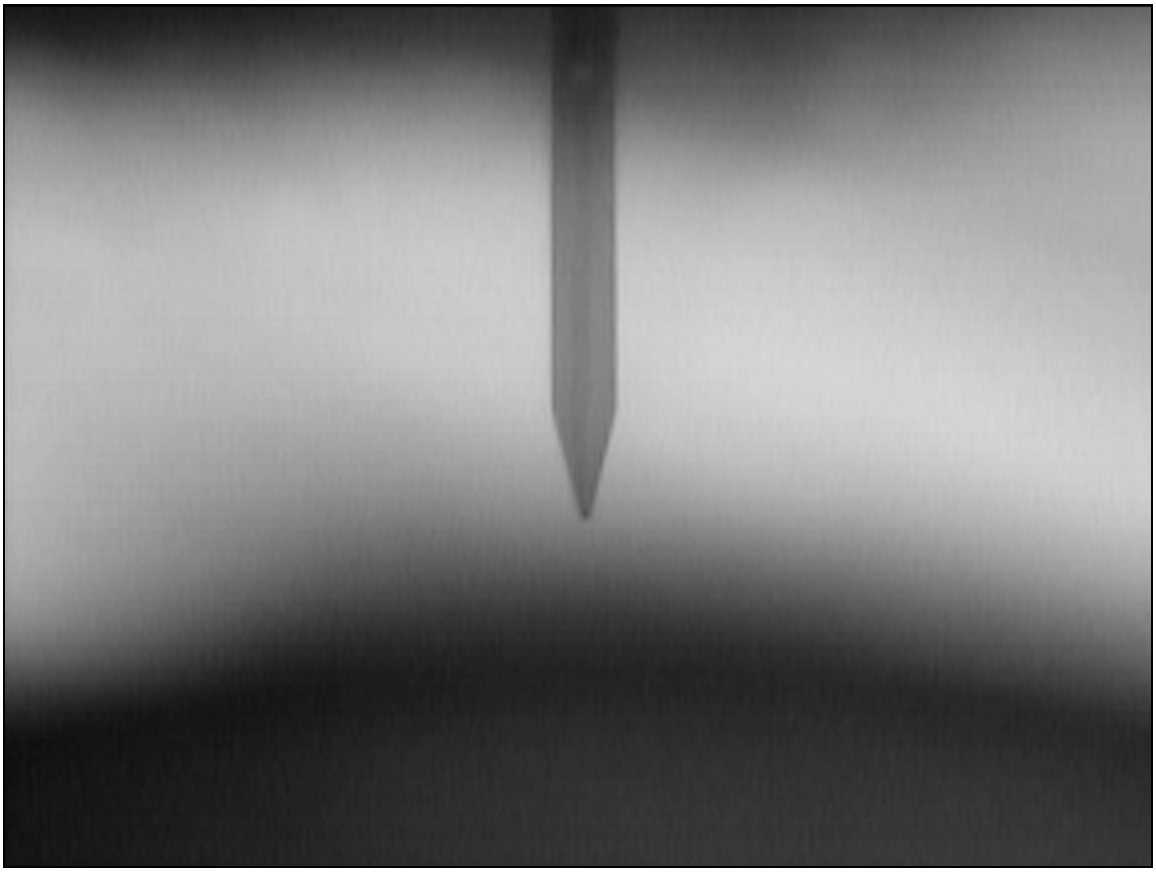
7. Blow dry the SPTs with Nitrogen (N_2) gas. Gas flow should be in the same direction as the SPT cantilevers to avoid cantilever breaking. Store SPTs in a clean container.

8. Pre-treat with UV/Ozone in the BioForce TipCleaner™ for at least 30 minutes immediately prior to use.

Washing the SPT while still mounted in the Nano eNabler can be a useful method to facilitate multiplexing without removing the SPT and re-aligning it to the previous position. Manual washing with a drop of ddiH_2O should be sufficient. Place a coverslip with a 1 μl drop of ddiH_2O on the sample platform.

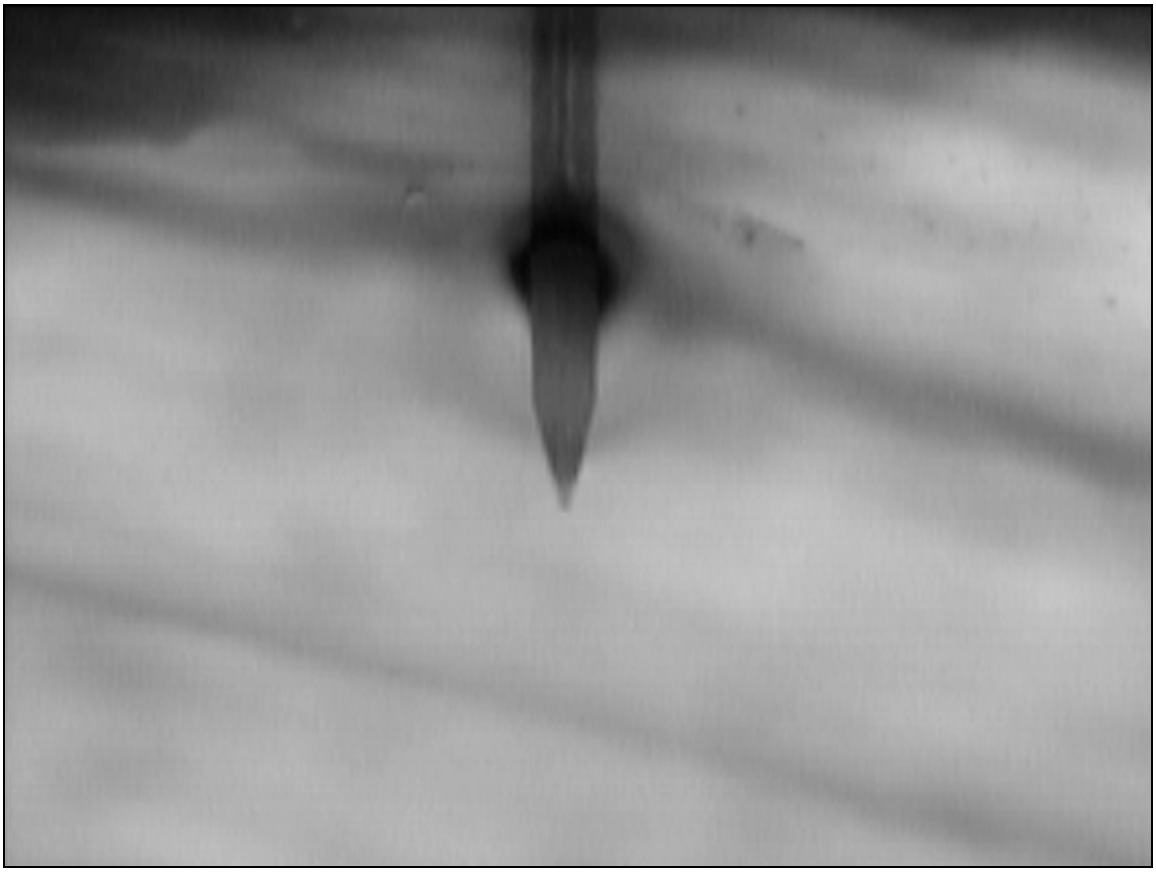


Maneuver the stage to position the edge of the droplet under the SPT.



Focus on the SPT and use the Coarse Z controls to bring the SPT down near the surface of the coverslip adjacent to the drop, but not in it.

Use the X-Y Stage controls to carefully immerse the cantilever.



Beware the capillary wicking of water trapped between a cantilever and the coverslip surface. This can lead to improper washing as well as wetting of the entire silicon substrate. Allow the cantilever to rinse for a short period of time, with occasional agitation by translating the X-Y Stage. To end the wash, simply raise the Coarse Z Stage to pull the cantilever out of the drop.

3.3.11 Shutdown

Sequence of Events

- Raise Coarse Z Stage to Load position and bring X-Y Stage to Load position 

- Remove surfaces from the sample platform
- Press “Exit” Button in NanoWare™ software 

- Turn the dry, inert gas valve off
- Shut down computer

4 NanoWare™ Software — The Graphical User Interface

4.1 Starting The NanoWare™ Software

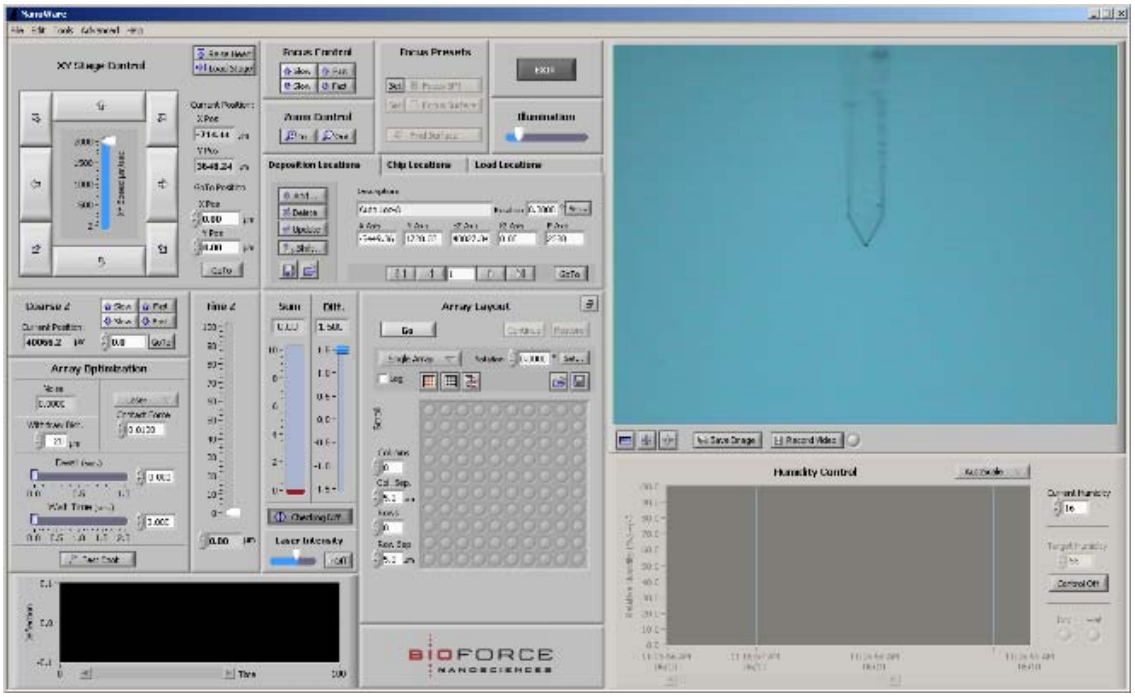
4.2 Introduction to The NanoWare™ Software

4.3 Miscellaneous Features

4.1 Starting The NanoWare™ Software

4.1.1 The Control Window

Power on the Nano eNabler™ Controller by pressing the button on the front of the rack. Turn on the monitor. After Windows loads, start the NanoWare™ software by double-clicking the “BioForce Nano eNabler” icon on the desktop or under the Start… Programs menu.



4.2 Introduction to The NanoWare™ Software

4.2.1 Overview

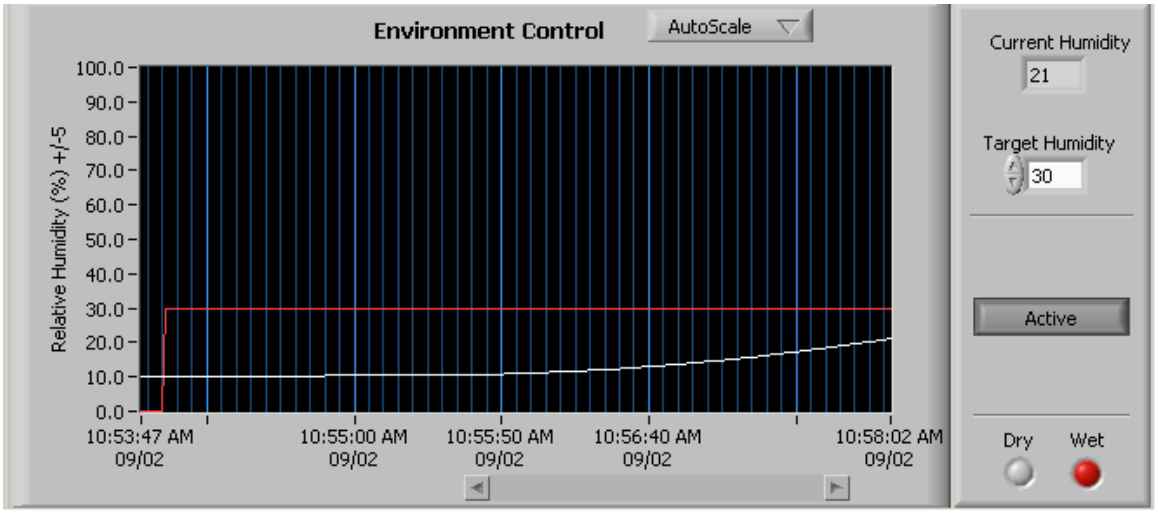
Adjust Environment
Mount SPT onto tool holder
Find SPT optically
Align laser
Adjust sum and difference
Set focal planes, find surface
Test Spot and Writing

4.2.2 General Environmental Control

The Nano eNabler's environment within the main chamber is controlled via the software and is fully adjustable to suit the needs of a particular situation.

Humidity Chart

Operation of the general environmental humidity control is controlled by the Active button in the Environmental control panel in the lower right corner of the NanoWare™ software interface. When the Active button is selected, the Nano eNabler™ will attempt to maintain the target humidity.



Deactivating the environmental control system is accomplished by depressing the Active button to display Control Off. The environmental control system will no longer attempt to regulate the humidity inside the chamber. This feature is useful if you want to take a

break and walk away from the Nano eNabler™ on a humid day without wasting any research grade dry nitrogen.

The charting window displays the historical relative humidity within the environmental box since the program was started, as well as the past target values. This makes it easy to see the changes in the relative humidity and target values as a function of time. Actual relative humidity is plotted in white and the target value is displayed as a red line.

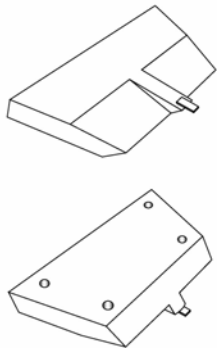
While the environmental controls can adjust the humidity levels to some degree, there are limits as to how high or low the environmental humidity can go. This limitation results primarily from the strong influence of the room environment.

Setting Target Humidity

Setting the target humidity is accomplished by typing the desired relative humidity value into the Target Humidity box below the humidity chart.

4.2.3 Instrument Setup

Mounting the SPT



The Nano eNabler™ monitors deposition events through feedback from an optical lever system. For proper function, the SPT must be mounted correctly. The SPT must be placed such that approximately 1-2mm protrudes past the tip holder. The cantilevers should protrude past the edge of the holder. The SPT must also rest flat against the holder. If the SPT substrate is tilted it may be difficult or impossible to get a usable reflective signal from the laser. Once the SPT is mounted in the holder, solutions may be back-loaded in the reservoir(s) by pipette.

Once the solutions are loaded onto the SPT, the holder may be mounted on the Nano eNabler™ Head. Mount the tip holder by sliding it into the head insert until the magnets lock it in place.

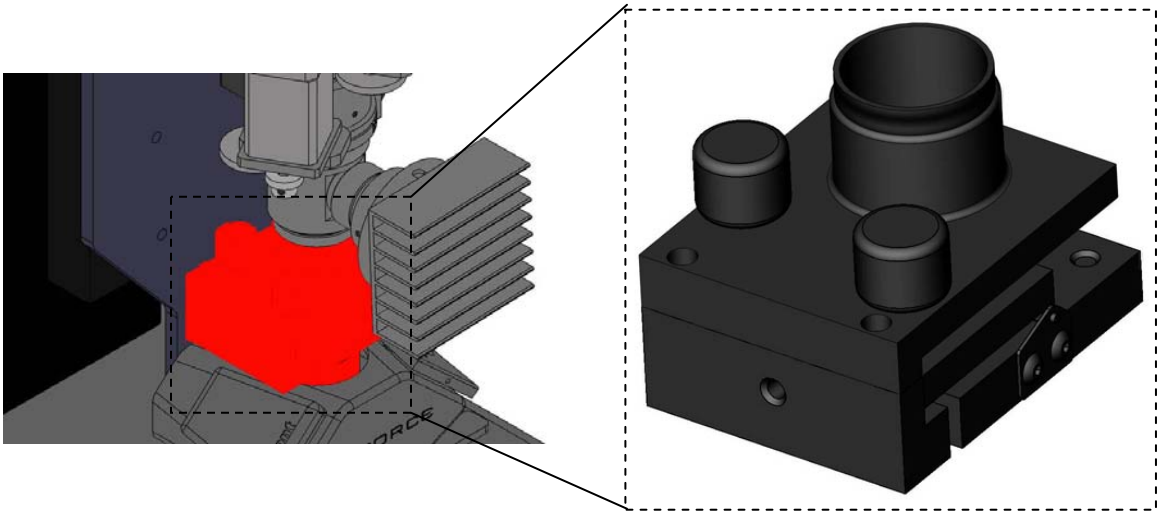
Finding the SPT Optically

Once the SPT and holder are mounted on the head, the optical system may be adjusted to observe the SPT.

First check that:

1. The LED illumination light is slightly dimmed
2. The Optical Zoom is set to its minimum.

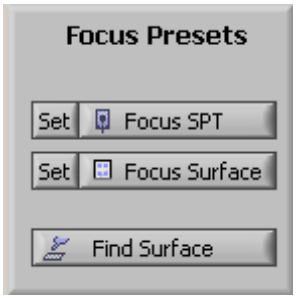
Finding the SPT optically is a balance between adjusting the focal plane and adjusting the X-Y position of the optics. The focus position is controlled from within the NanoWare™ software. The X-Y position of the optical system is controlled by two adjustment thumbscrews near the bottom of the optical stack. These thumbscrews allow the objective to pivot towards SPT and center it within the on-screen video.



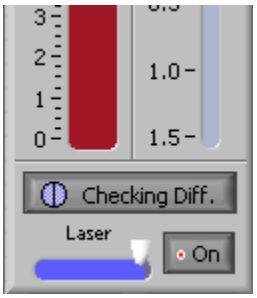
Ensure that the zoom is set to the lowest magnification by pressing and holding the Zoom Out button. Locating the SPT at a high magnification zoom setting can be very difficult. Adjust the X-Y positioning of the optical system with the X-Y optics thumbscrews while observing the on-screen video image. Adjust focus to sharpen the image as soon as objects are observed entering the field of view. If the silicon substrate of the SPT is found, follow the lines of the substrate to find the cantilever. Center the end of the cantilever in the field of view and adjust the focus to sharpen the image.

Set SPT

Once the SPT is focused, click the SET button next to the Focus SPT button. This sets the SPT surface plane to memory.



Aligning the Laser

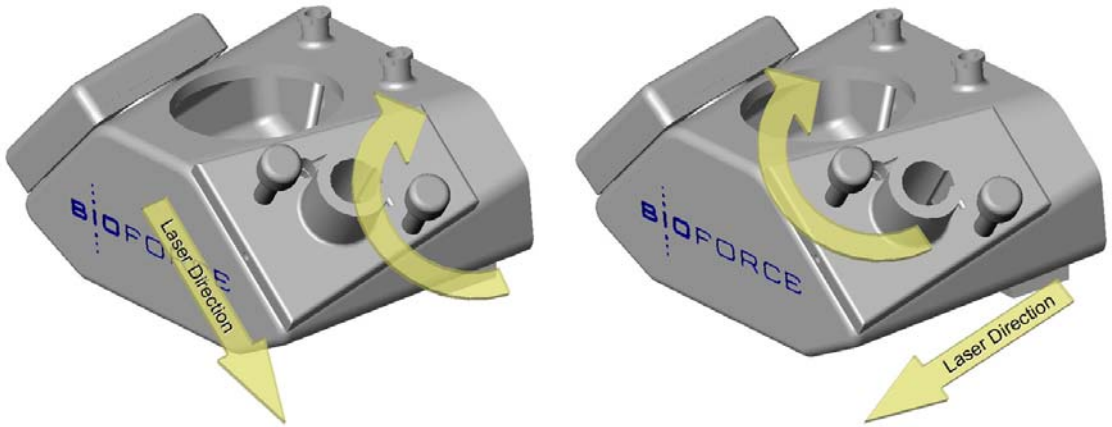


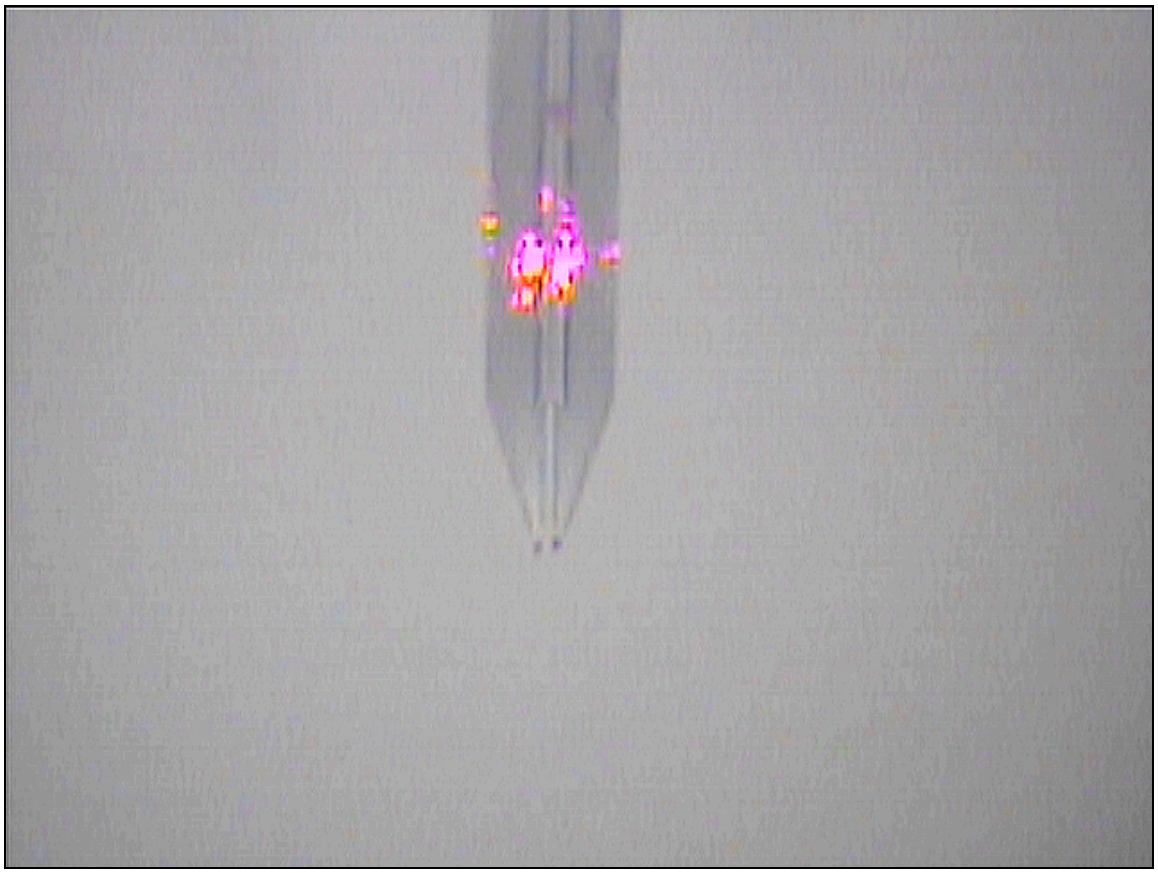
Positioning of the laser is controlled by two knobs on the right side of the Nano eNabler™ head. These thumbscrews tilt the laser mount relative to the head and move the focused laser beam position. Proper placement of the laser beam on the SPT is required for force feedback. The laser will initially be turned on. To toggle the laser on or off, press the On/Off Button labeled Laser, beneath the Sum and Diff. difference displays.

Rough initial positioning of the laser is often most easily accomplished by the naked eye rather than using the optical microscope.

WARNING: LASER HAZARD. Exposure to direct or redirected laser light can burn the retina and leave permanent blind spots. Never look directly into the laser beam.

Move the thumbscrews back and forth until you find the laser beam striking a solid surface such as the silicon substrate of the SPT or the SPT holder. Continue to use the thumbscrews to move that beam into the field of view of the optical microscope at its lowest magnification. Decrease or increase the intensity of the LED illumination, as necessary, to make the laser easier to visualize on-screen. Position the laser beam on the cantilever using the two thumbscrews. The system will be most sensitive with the laser beam focused 2/3 from the base of the cantilever. Moving the laser beam down the cantilever away from the end will reduce sensitivity due to the decreased cantilever deflection at that point.



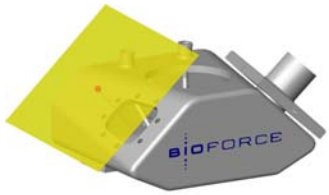


Optimizing the Photodetector Position

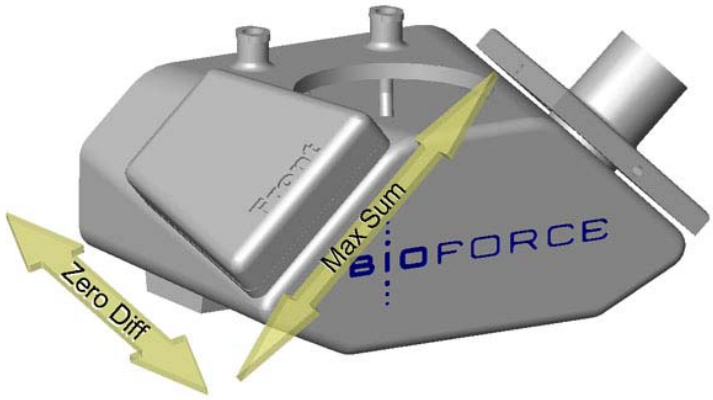
The photodetector must be properly positioned for optimal deflection sensitivity. This can be accomplished by manually sliding the magnetically held photodetector puck against the magnetic surface on the Multi-Component Head. Raise the Multi-Component Head using the Coarse Z Stage controls until it is several millimeters above the surface. Turn the laser on by clicking the Laser button underneath the Sum and Diff. indicators in the NanoWare™ software. The green LED on the end of the laser should turn on and the red laser beam should be visible on the video screen. Ensure that the laser beam is striking the end of the SPT for maximal sensitivity. Remove the photodetector from the Multi-Component Head to expose the hole through which the laser beam passes.

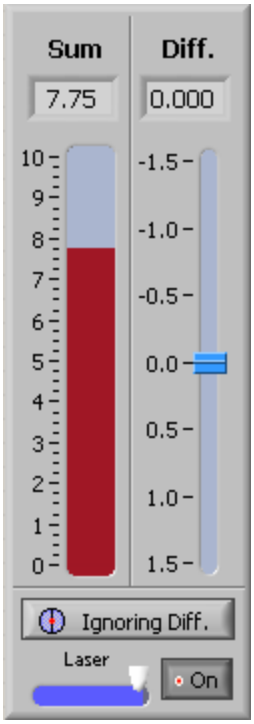
WARNING: LASER HAZARD. Exposure to direct or redirected laser light can burn the retina and leave permanent blind spots. Never look directly into the laser beam.

Place a piece of paper, such as a Post-It Note over the hole to check the positioning of the laser beam. It should be roughly centered in the hole if the SPT is positioned properly in the SPT holder.



If the laser beam is badly off-center or striking the edge of the hole you must first adjust the angle of the SPT. If the laser is striking the front edge of the hole, that is an indication that the angle of the cantilever is too steep. This may be a result of an improperly positioned SPT, or a sign of a warped cantilever. If the laser is striking the back edge of the hole, then that would indicate that the cantilever angle is too flat. Adjust the angle by placing a tiny piece of Double-sided Stick Tape underneath the front or back edge of the SPT substrate as a shim. Fine-tune as necessary to center the laser beam in the hole. Remove the piece of paper and replace the photodetector in the correct orientation.

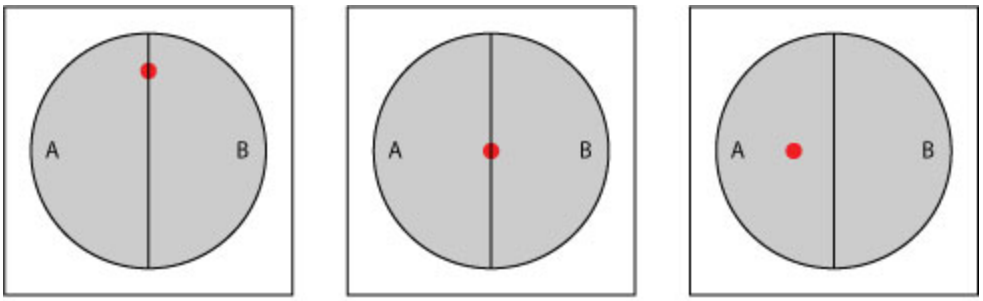




Monitor the Sum and Difference indicators in the NanoWare™ software as you slide the photodetector from front to back.

When the laser is centered on the photodetector it should produce the highest possible Sum value (A+B) on the vertical indicator. Moving the photodetector up and down against the head should maximize this value. If a Sum close to 10 is reached, attempt to center the photodetector in the middle of the positioning range that gives that value. After maximizing the Sum, slide the photodetector forward and backward to adjust the Difference to a value of zero as indicated by the numerical display above the vertical indicator. This is actually a normalized Difference value as given by (A-B)/(A+B).

As depicted in the illustration below, the photodetector puck should be positioned such that the laser beam strikes the center of the photodetector. The first example is incorrect because the Sum is likely not maximized, and the path of the beam will move off of the photodetector as the SPT deflects. The third example is incorrect because although the Sum may be high, the laser spot is not evenly divided between the A and B halves of the photodetector. The center example depicts a properly positioned photodetector, with the laser centered vertically for maximum Sum and laterally for a zeroed Difference.



NO YES NO

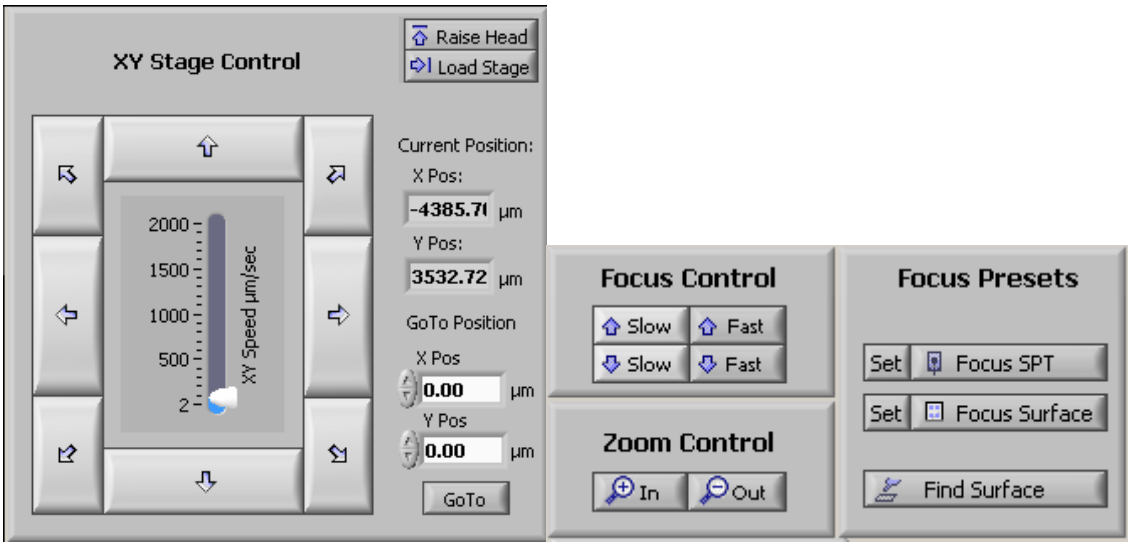
While you are working, the Difference value may drift away from zero and should be manually re-adjusted as necessary. If the Checking Diff. button is selected, a dialog box will pop up and suggest that you make an adjustment any time that the Difference drifts out of the optimal working range.

To disable this feature, click the Checking Diff. button so that it shows Ignore Diff.

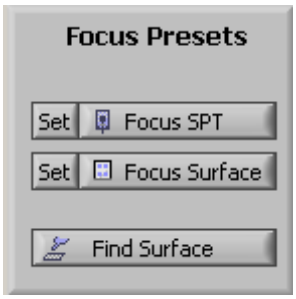
Although the dialog box will not be popping up to remind you, it is still important to keep the Difference close to zero for maximum sensitivity. After optimizing the position of the photodetector, re-tune the positioning of the laser for best results.

Set Surface

Click on the Course Z down fast button to move the SPT to a distance of approximately 2-3mm above the substrate surface. Move the substrate into view of the optics by clicking on the XY-stage control. Focus down onto the surface and click on the SET button next to the Focus Surface button, which sets the surface plane to memory.



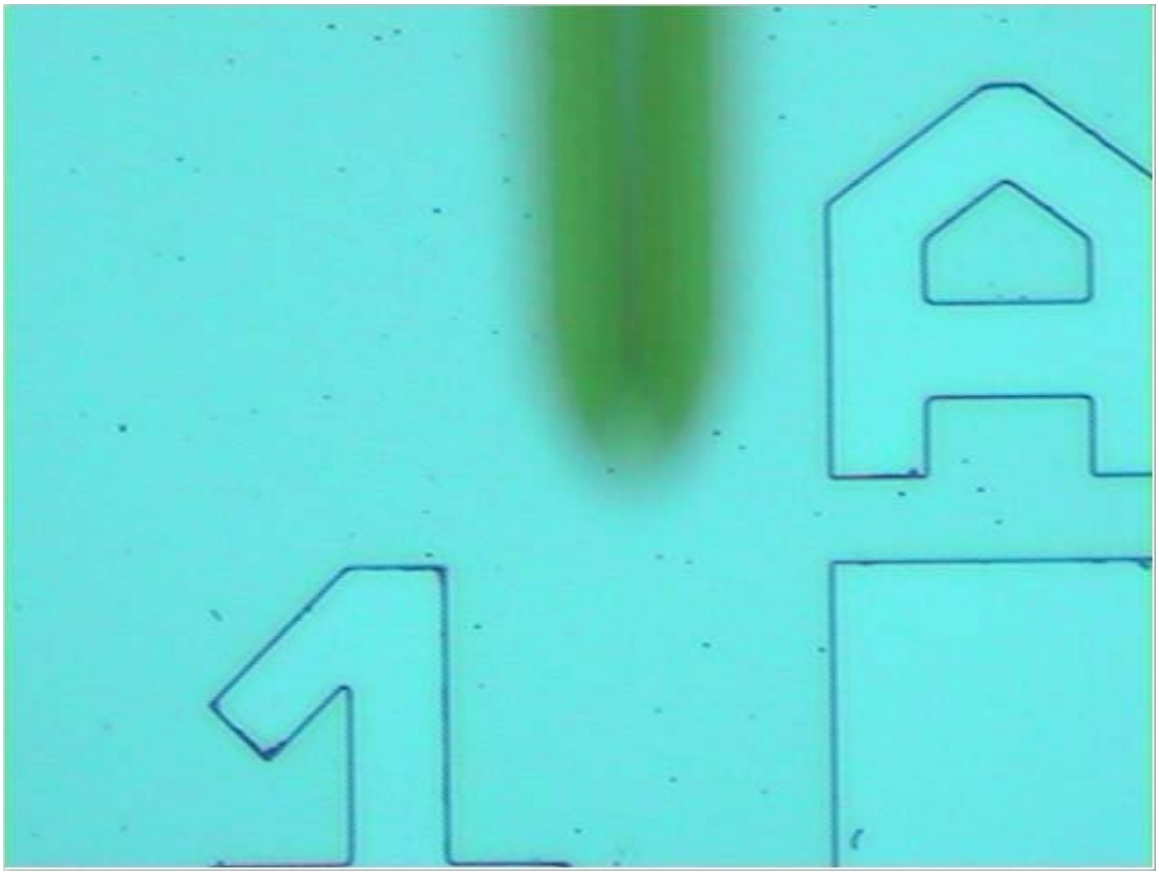
Finding the Surface



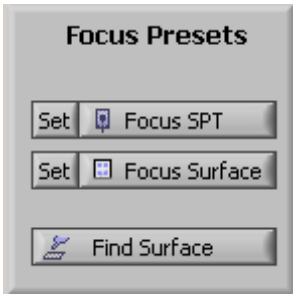
Press the Find Surface button. The optical system will focus down to its lower limit, use that as a reference point, and then focus back up to the designated plane. Due to backlash and other mechanical factors, the optical system may not return to the correct focal plane after you press Set. Re-adjust the focus and press Set again. Repeat until a

satisfactory focal plane is achieved. Use the Focus Tool button any time that you wish to focus on the cantilever.

Keep in mind however, that unlike the SPT, the focal plane of the surface will change as the position of the Fine Z Stage changes. The Focus Surface button does take into account movements on the Z axis and attempts to keep the surface plane in focus.



With the SPT and surface focal planes now defined, the Nano eNabler™ can engage the surface by itself.

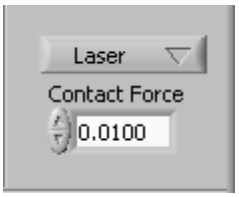


When you press the Find Surface button, the optical system will focus on the SPT and bring the Multi Component Head down to within 100-200 µm of the surface using the Coarse Z Stage. At this point the high precision Fine Z Stage takes over and raises the surface up to the SPT until either a contact event is registered by the laser/photodetector

system, or the Fine Z Stage runs out of travel. In that case, the Coarse Z Stage moves down and the Fine Z Stage makes another attempt. Eventually a deflection of the cantilever is detected, and the Fine Z Stage lowers the surface by the distance specified in the Withdraw Distance box. This initial process of engaging the surface takes only a few seconds.

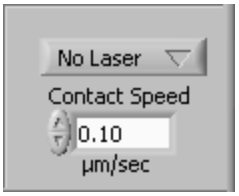
Laser Feedback

In Laser Feedback mode a numerical entry box will appear below the Laser button. Enter the desired contact force in that box. You may need to run the Test spot routine a few times to fine-tune the contact force. If the value is set too low, the system noise may cause a false engage. If the contact force value is set too high, the SPT may smash into the surface. A value of 0.01 and moving up to a maximum of 0.03 is suitable for most applications.



No Laser Feedback

The No Laser Feedback mode uses no feedback mechanism. Use this mode after you are at a known distance from the surface. Using this mode the Fine Z stage will simply move up the withdraw distance, pause for the dwell time, and withdraw again. Because the feedback mechanism is disabled in No Laser mode, the printing process is significantly faster.

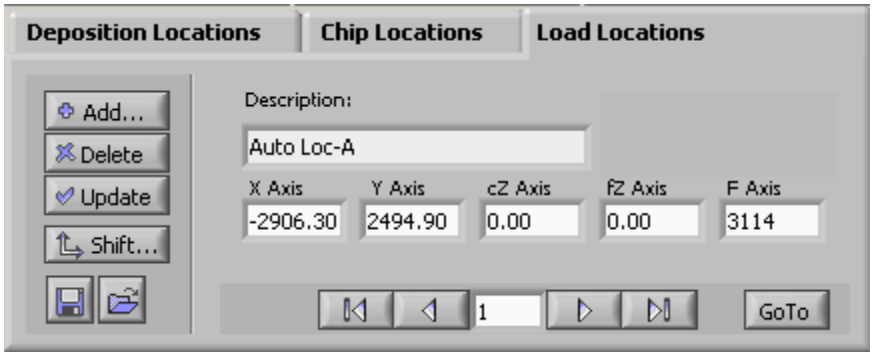


4.2.4 Using Preset Locations

Preset locations allow navigating to a position only once and allowing the software to bring the tool back to that location in future moves. There are three tabs where preset locations may be saved, the Chip Locations, Deposition Locations, and Load Locations. Chip Locations are used to store the general location of a Chip, Deposition Locations are used for printing on multiple chips (see Printing Multiple Arrays, 4.2.6), and Load Locations are used to store the location of front-loaded printing materials.

Adding and Deleting Load Locations

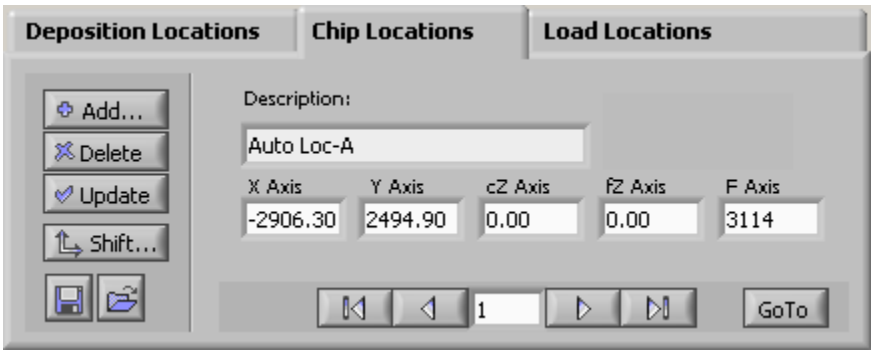
Maneuver to the first desired load location and engage the surface. Immerse the SPT as desired for loading by adjusting the X-Y Stage and Fine Z Stage position. Select the Load Locations tab to bring it forward.



Press the Add button, enter a description, and you will see the current X, Y, Coarse Z, and Fine Z positions all recorded along with your description. Each location is also assigned an index number that is displayed at the bottom. These numbers indicate the order in which locations were added, as well as the order in which they will be browsed. Use the navigation arrows around each index to navigate through saved Load Locations.

Adding and Deleting Chip Locations

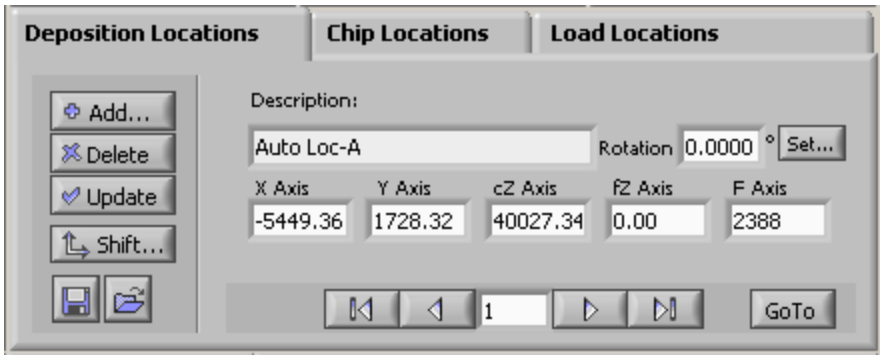
Maneuver to the first desired chip deposition location and engage the surface. This position can be anywhere on the chip to help locate it again. Select the Chip Locations tab to bring it forward.



Press the Add button, enter a description, and you will see the current X, Y, Coarse Z, and Fine Z positions all recorded along with your description. Each location is also assigned a number that is displayed in the lower left corner. These numbers indicate the order in which locations were added, as well as the order in which they will be browsed. Use the navigation arrows around each index to navigate through saved Load Locations.

Deposition Locations

Maneuver to the first desired deposition location and engage the surface. This position will be the location of the first, lower left spot in the array. Select the Deposition Locations tab to bring that it forward.



Press the Add button, enter a description, and you will see the current X, Y, Coarse Z, and Fine Z positions all recorded along with your description. Each location is also assigned a number that is displayed in the lower left corner. These numbers indicate the order in which locations were added, as well as the order in which they will be browsed. Use the navigation arrows around each index to navigate through saved Load Locations.

Deposition Locations work in conjunction with the Multiple Arrays button in the array layout section. By adding the lower left locations to where each array should start and

toggling the Single array button to Multi-Array, when an array is executed it will write an array at each deposition location.

Moving to a Preset Location

Browse the locations using the arrows next to the location index number box. As you scroll through preset locations, the descriptions should be sufficient to identify the particular sample. Pressing the GoTo button to the right of Browse Locations will take you to the preset location that is currently being displayed. Note that during a GoTo move, the Z axis will raise up 1.5 centimeters in an attempt to clear any objects protruding from the XY stage.

Saving Preset Locations to a File

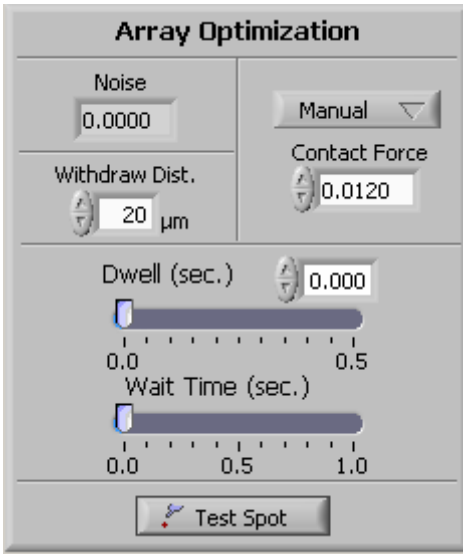
If you have added several preset Load Locations or Deposition Locations, it is possible to Save that list of locations to a file for later use. For users loading from a microarray format, this means only having to define the microarray loading spot positions once. The microscope slide containing the samples could be removed from the X-Y Stage for refrigerated storage, and reused with the same list of preset Load Locations as long as it was placed back on the stage in the correct position. In a production setting, the starting Deposition Locations for an entire X-Y Stage full of chips could be defined and stored to a file. After that batch of chips has been processed, fresh chips could be placed on the stage in the same locations for the next round of printing using the preset locations. This is also convenient should you be forced to close the Nano eNabler™ software or restart the computer during operation. Please note that you must save Load Locations and Deposition Locations independently from one another by clicking the Save button from each respective tabbed box. Simply saving the Deposition Locations will not also write the Load Locations to the same file.

Loading Preset Locations from a File

To use a group of preset Load or Deposition Locations that have been saved to a file, first position the loading slide or deposition chips on the X-Y Stage in the same position as when the preset locations were saved. Select Load from either the Deposition Locations tab or the Load Locations tab, and follow the instructions.

4.2.5 Deposition Control and Optimization

We have developed an instrument and a methodology for the reliable deposition of materials, including biomolecules. Due to all the potential variability between samples and applications, we find that optimization must be performed for best results. The Nano eNabler™ software interface has been designed to offer maximum control and flexibility for the end user.



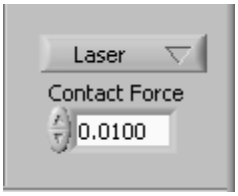
The Test Spot Button

After loading the SPT with sample the printing conditions need to be optimized using the Test Spot button. Find an area of the deposition chip away from where you intend to make the array. Press Test, check the spot size, using the on-screen measurement tool, make any necessary adjustments to the Dwell Time, Environment, etc and then press Test Spot again to evaluate the changes. Navigate to the desired location and start patterning when you are satisfied with the results.

Contact Force

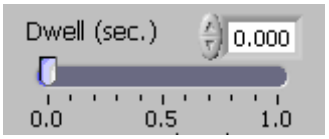
Contact Force is defined as the voltage change between the normalized Difference values taken at two successive time points. Deflection of the cantilever by the surface leads to a more negative Difference, while attractive forces result in a more positive Difference voltage. The changes between successive data points are displayed on the Contact Force Chart. During the portion of the approach before contact is made, a certain amount of noise will be visible in the chart. This noise level will depend upon the cantilever being used as well as the positioning of the laser and photodetector. If operating the Nano eNabler™ in Laser Feedback mode, the specified contact force should be selected such that it exceeds the amount of noise to prevent false engages.

The Noise value displayed with the laser turned on is usually a good starting point for determining the minimum Contact Force in manual mode. It is recommended to use the smallest Contact Force threshold possible that provides consistent depositions. Larger forces are possible and may be necessary in some instances; however they can lead to over-sized spots, as well as damaged surfaces and SPTs.



Dwell Time

Dwell Time is indicated both by a digital display box and with a slider. Changes made to either one will automatically be reflected in the other. All times are represented in seconds, with Time 0.0 being the instant when a surface contact event is detected. It is possible to enter a Dwell Time in the digital display box that exceeds the display range of the slider scale. Adjustments can be made to the Dwell Time slider scale by double-clicking the number at the far right end of the slider and typing a new maximum. Start with a short Dwell Time such as 0.0 or 0.1 seconds and gradually increase the time to adjust the amount of material transferred to the surface. A Dwell Time of 0.0 seconds is actually an acceptable setting, as it represents the minimum amount of time it takes the computer to process the contact event and send the signal to withdraw the Fine Z Stage. The optimal Dwell Time will depend upon the concentration of the material being deposited, the viscosity of the solution, and the relative humidity inside the environmental chamber.



Withdraw Distance

The Withdraw Distance specifies the distance in microns for the Fine Z Stage to pull back after making each spot. This can be adjusted from 0 to 100 µm depending on the stiffness of the SPT being used and the distance from the tool to the surface. A stiff cantilever will require less Withdraw Distance to break the capillary bridge with the surface. Thirty microns is a good starting point for most cantilevers. This value can be adjusted as necessary to aid in breaking free from the surface, or reduced to shorten the amount of time required for each spot. Large Withdraw Distances may not always be possible, as this will depend upon the position of the Fine Z Stage at the point when the Find Surface routine detected cantilever deflection. Create room for large Withdraw Distances by moving the Coarse Z up slowly so that the Fine Z Stage does not bring the surface into contact with the SPT until somewhere between 90 - 100 µm.

Reducing to a smaller Withdraw Distance of 20 µm or less will slightly speed up the deposition process; however it might not be sufficient for use with highly viscous samples and/or weaker SPTs.

A larger Withdraw Distance of 75 µm may enable the use of weaker cantilevers and/or more viscous samples; however there may not be 75 µm of travel available for Fine Z Stage withdrawal unless you first manually raise the Multi-Component Head with the Coarse Z Stage. To test this, first turn the laser on, put the Fine Z Stage in Manual mode, and then raise the Fine Z slider until cantilever deflection causes a shift in the Difference signal. Make a note of the position on the Fine Z slider where this contact event occurs. That point will be the maximum Withdraw Distance since the Fine Z Stage can only pull back to zero. If you need a greater Withdraw Distance, raise the Coarse Z Stage either with the Coarse Z Up Slow button or directly by entering a desired position into the Coarse Z box and pressing GoTo. Re-check the point of surface contact using the Fine Z slider to ensure that you have the preferred amount of Withdraw Distance available and that you are still able to contact the surface.

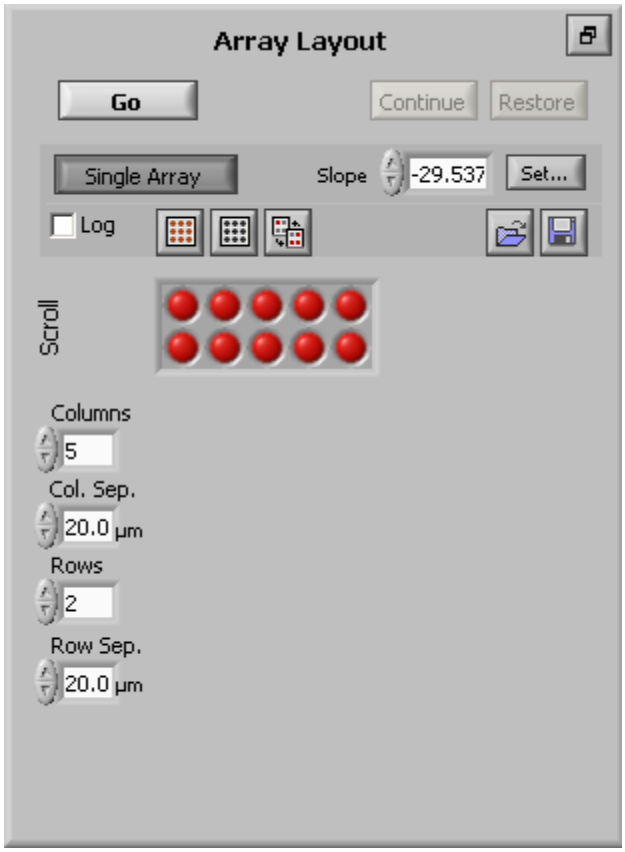
Wait Time

Wait Time is defined as the interval immediately following the withdrawal of the Fine Z Stage but before the translation of the X-Y Stage to the next location. This slider is normally left at 0.0 seconds, but can be increased to allow extra time between spots if necessary. Similar to the other sliders, the scale can be modified by double-clicking on the maximum value and typing in a new maximum.

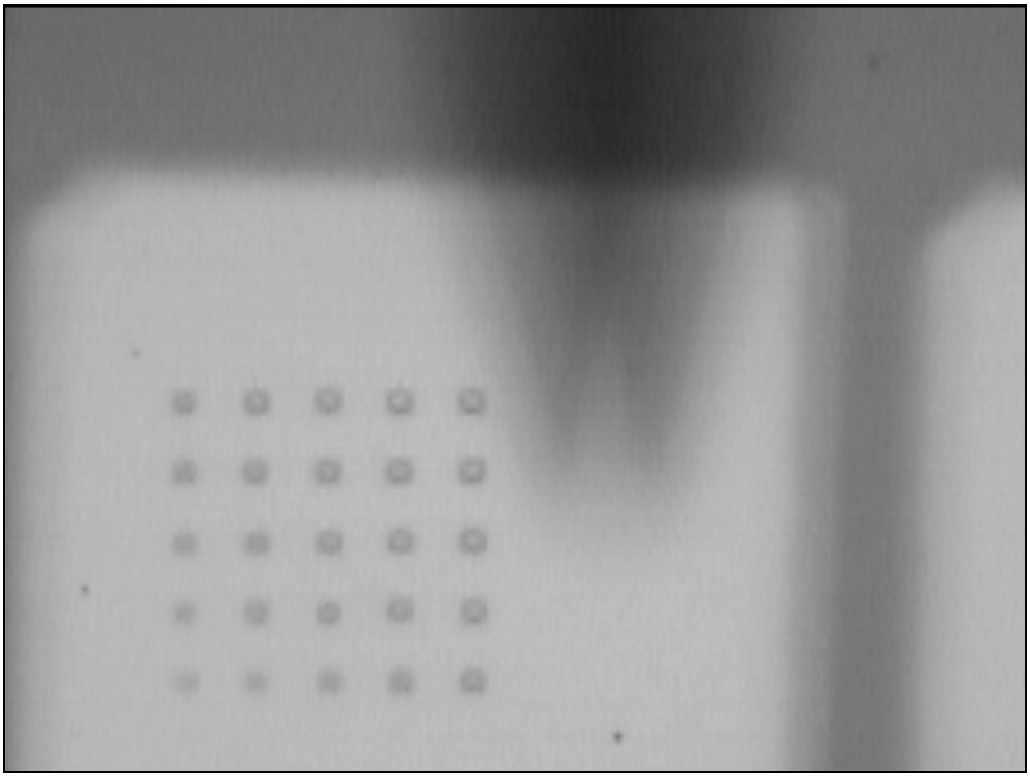
4.2.6 Deposition Format

Printing Arrays

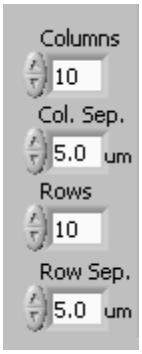
The Nano eNabler™ software package makes it easy to design and create complex patterns. The Array Layout window features an intuitive interface and an interactive display that is updated in real-time as the array is being printed.



Go from design concept to completed array in just minutes!

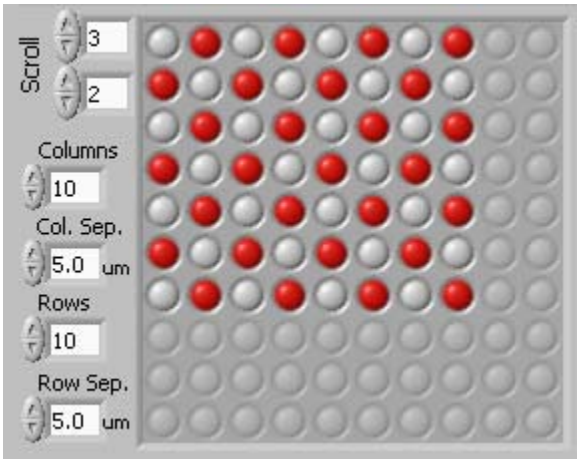


Custom Array Design



The Nano eNabler™ software is a powerful, yet simple, tool for creating your own custom array designs. First, specify the desired number of Columns and Rows by entering each number into its respective box.

If you select a number of Columns or Rows greater than 10, a control will appear to aid in scrolling. As you scroll to the edges of the array, the surrounding spots will be grayed out to denote the boundary.



Then specify the desired Column or Row Separation (in microns) in the boxes to the right. This will obviously depend upon the diameter of the spots being deposited. Larger spots require greater spacing to prevent merging. Careful adjustment of the deposition parameters will allow creation of smaller spots and higher density arrays. Once the grid of columns and rows has been set up, it is time to select the spots to print. Red circles indicate a spot that will be printed, and grey circles indicate a spot that will be skipped. Click on any spot to toggle between these two states.

Use the “All On” button () to make all of the spots red, and the “All Off” button () to turn all of the spots grey. The “Invert” button provides a convenient method for reversing the state of every spot in the array (). This is useful when interdigitating spots of two different materials in the same array.

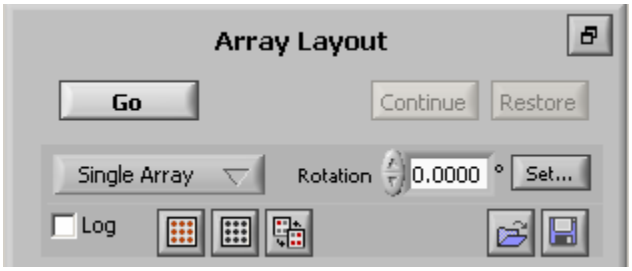
Explore the flexibility to create patterns, write words, or anything you can conceive!

Saving and Loading Array Patterns

Use the Save Pattern () and the Load Pattern () buttons to avoid having to recreate complex array patterns. Build up and save a library of frequently used patterns for easy retrieval and re-use.

Printing Single Arrays

Two modes of printing have been developed. The first mode is referred to as “Single Array” mode and it is well suited for writing one array on one chip at a time. This is often sufficient for many one-off experiments that are testing a concept or require direct user control. Pressing “Go” while in “Single” mode will result in the printing of a single array, starting at the current location and following the pattern that has been set. Select this mode by pressing the “Single Array / Multiple Arrays” button until it displays “Single Array”.



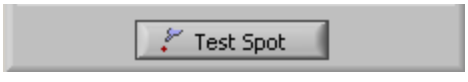
Printing Multiple Arrays

The second mode of printing is “Multiple Arrays” mode and it was designed to facilitate the creation of identical arrays on multiple chips much like the operation of a microarrayer. Pressing “Go” while in “Multiple Array” mode will print each spot selected in the array pattern at each preset Deposition Location tab.

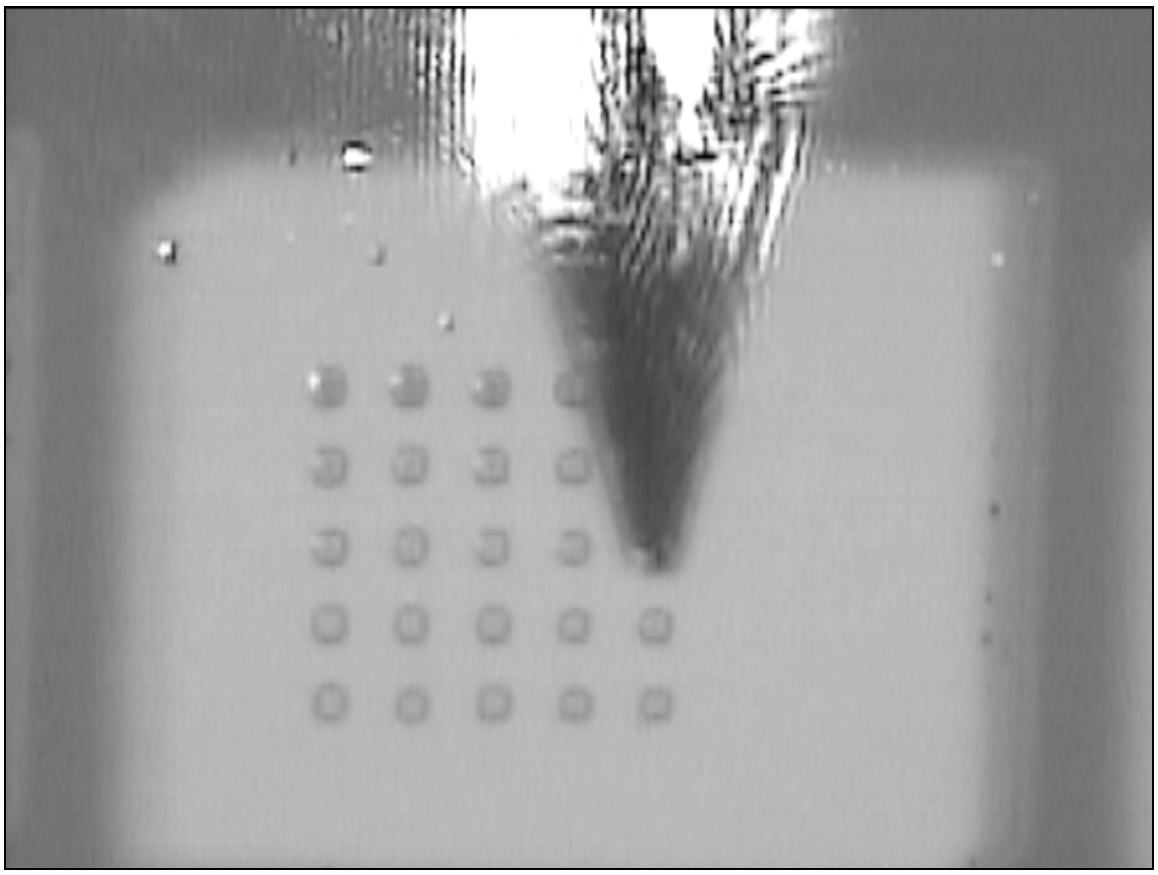
4.2.7 Patterning Process

Before You Start – The Test Spot Button

After loading the SPT with sample you should optimize the printing conditions using the Test button. Find an area of the deposition chip away from where you intend to make the array. Press Test Spot, check the spot size, make any necessary adjustments to the Dwell Time, environment, etc and then press Test Spot again to evaluate the changes. Navigate to the desired location and start patterning when you are satisfied with the results



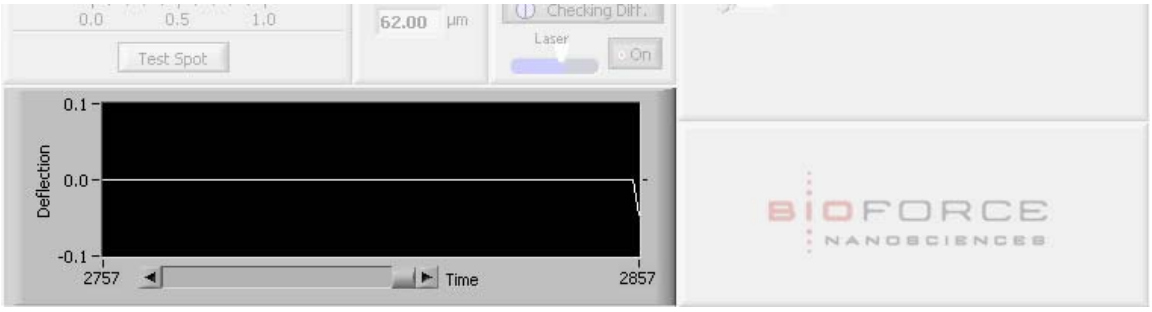
During an Array



Monitoring Contact Force Chart

The bottom left corner of the Nano eNabler™ software displays the Contact Force Chart after making each spot. This chart displays the cantilever deflection as indicated by changes in the Normalized Difference from the photodetector. A normal Contact Force chart should look like the one shown below. During the approach there will be a relatively flat portion that contains some system noise inherent in the cantilever and/or laser and photodetector positioning. At the point of contact the line will begin to slope down to indicate surface deflection. Occasionally the line will slope upward to indicate that the cantilever is being pulled down to the surface by attractive forces indicative of a thick layer of water on the surface.

Monitor the amount of noise in the flat portion of the line relative to the amount of deflection as a means of evaluating the Contact Force in manual mode. If the Contact Force threshold is set too low then a spike in the noise may be detected as deflection. Examining the Contact Force Chart should help diagnose whether the contact was real or false. A slow gradual increase or decrease is likely to trigger a false contact event. This can be the result of optical interference with highly reflective printing surfaces and an improperly positioned laser.



Monitoring Array Progress Display

While an array is in progress, you can monitor the status in the Array Layout window. As each spot is made it will turn from red to gray.

Adjusting Deposition Parameters

Several deposition parameters can be adjusted on-the-fly during the creation of an array, while a few require that the array be interrupted and then restored. Dwell Time, Wait Time, and Contact Force an all be optimized without pausing. Focus and Zoom positions, and Withdraw Distance can only be modified by first interrupting the array, making the changes, and then continuing with the array.

Interrupting an Array

Pause the creation of an array by pressing and holding the Stop Arraying button shown below. Click the Stop Arraying once and as soon as the software finds a consistent state to stop in, it will stop. Once the patterning process has halted you can make any necessary changes to the deposition parameters.

Continuing an Array

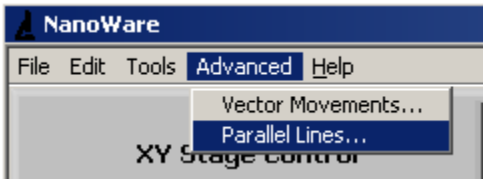
Once an array has been interrupted, for whatever reason, the user has the option to continue where the patterning stopped. The coordinates of each spot are determined when the Go button is pressed, so you can move to a different area and make a few Test spots, then press Continue and the Nano eNabler™ will automatically move back to the next spot in the array.

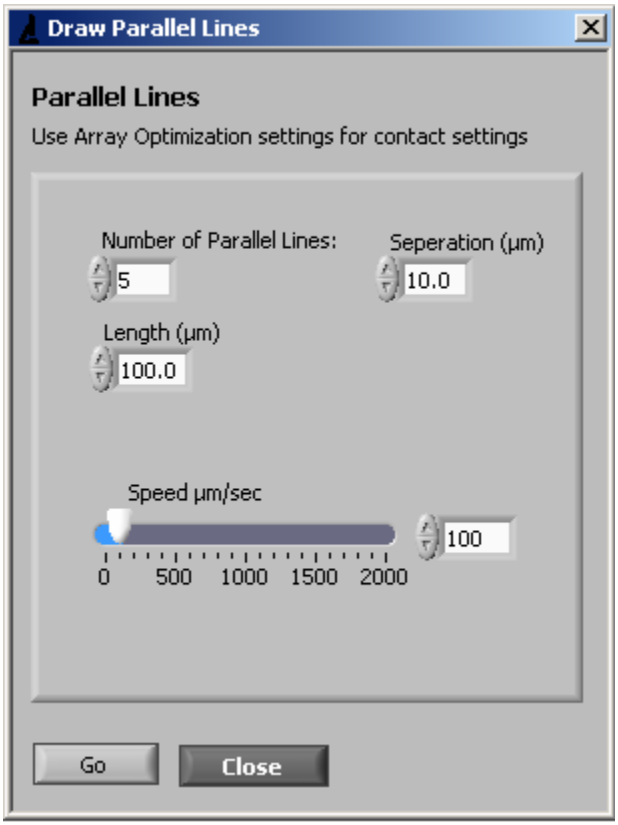
Restoring an Array

If array creation has been interrupted and you do not wish to complete the array, press the Restore button to return Array Layout to its state at the beginning of the patterning process.

4.2.8 Parallel Lines and Vector Movement

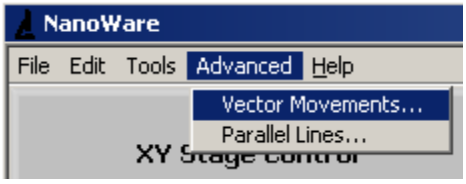
The Parallel lines can be created by clicking on the Advanced drop-down menu.



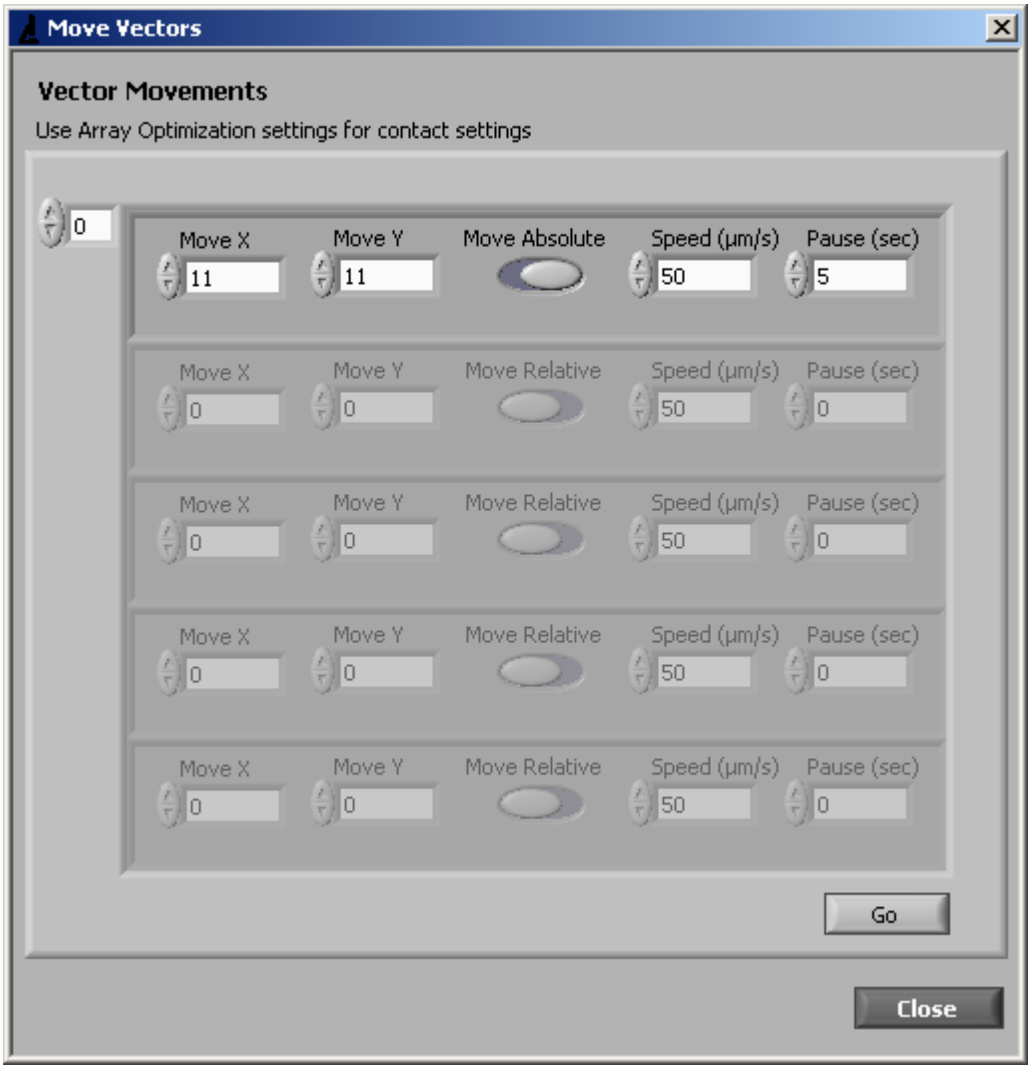


A pop up window allows for users to input the number of lines, the distance between the lines, length of the line, and speed.

The Vector movement, a almost “free-hand” drawing approach to create lines using X and Y coordinates. This feature is used by clicking on the Advanced drop-down menu



A window pops up to allow input of the X distance, Y distance, the speed, and the dwell time before movement.

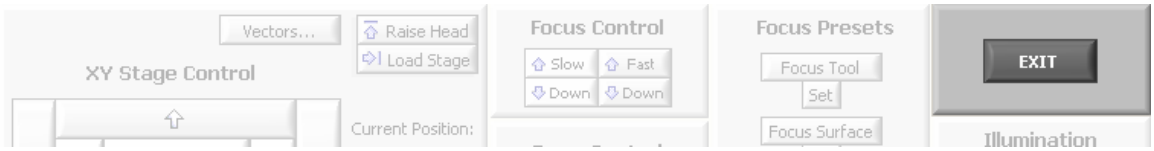


For example, to create a 25µm square box, the user would input:
Row 1, X=25um
Row 2, Y=25um
Row 3, X= -25um
Row 4, Y= -25um

A simultaneous input of x and y commands on the same line will generate a line drawn at a 45 degree angle.

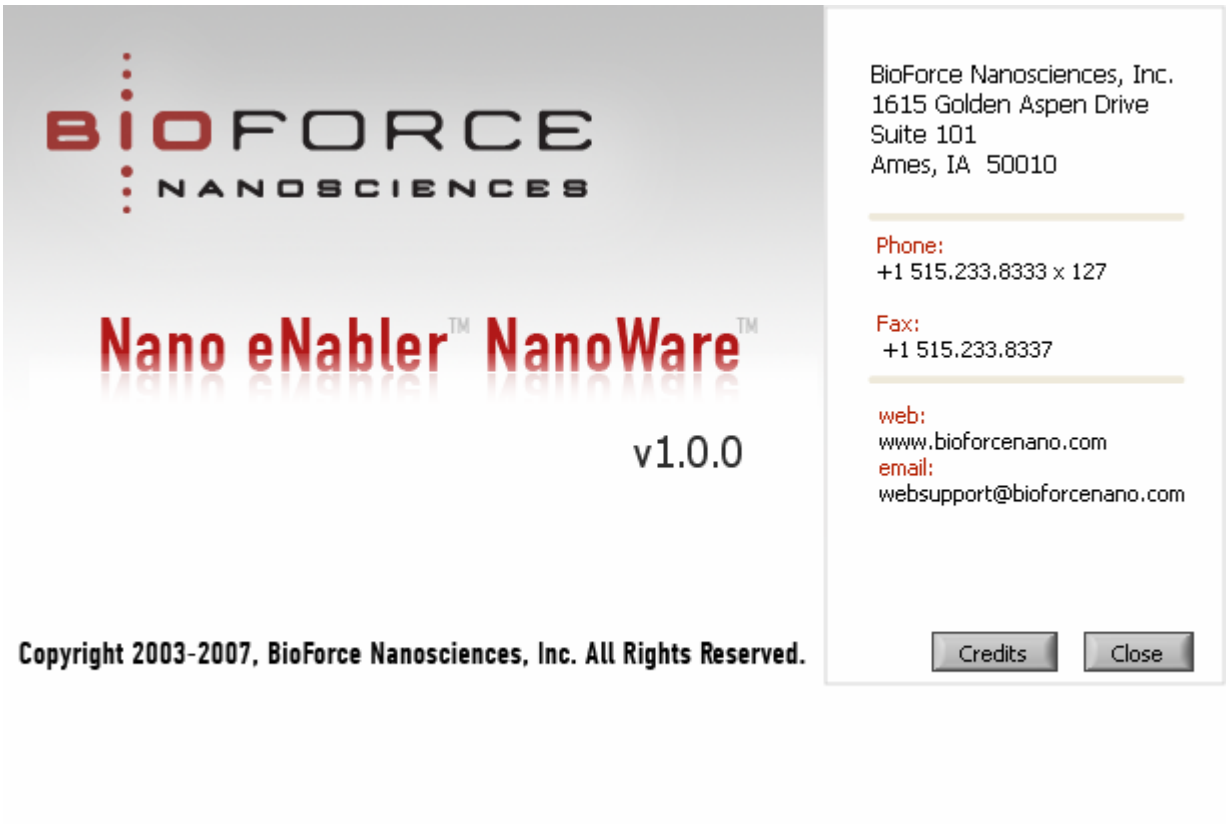
4.2.9 Shutting Down

Proper shut down of the NanoWare™ software is as simple as pressing the red STOP Program Execution button in the upper right corner of the user interface.



4.3 Miscellaneous Features

Clicking on the BioForce logo in the lower right corner of the user interface brings up an "About" box containing the version number and contact information for BioForce Nanosciences.



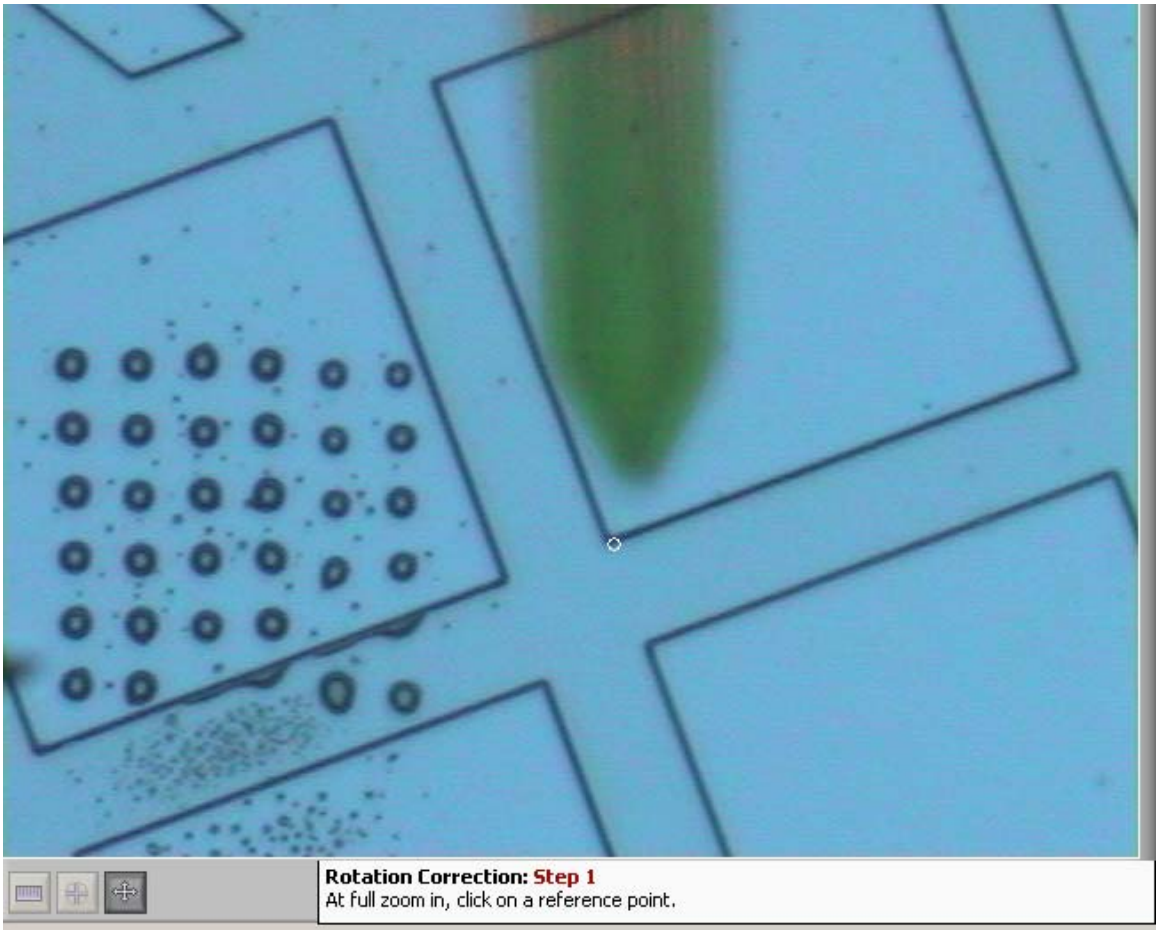
5 Advanced NanoWare™ Software Features

5.1 Array Rotation Correction

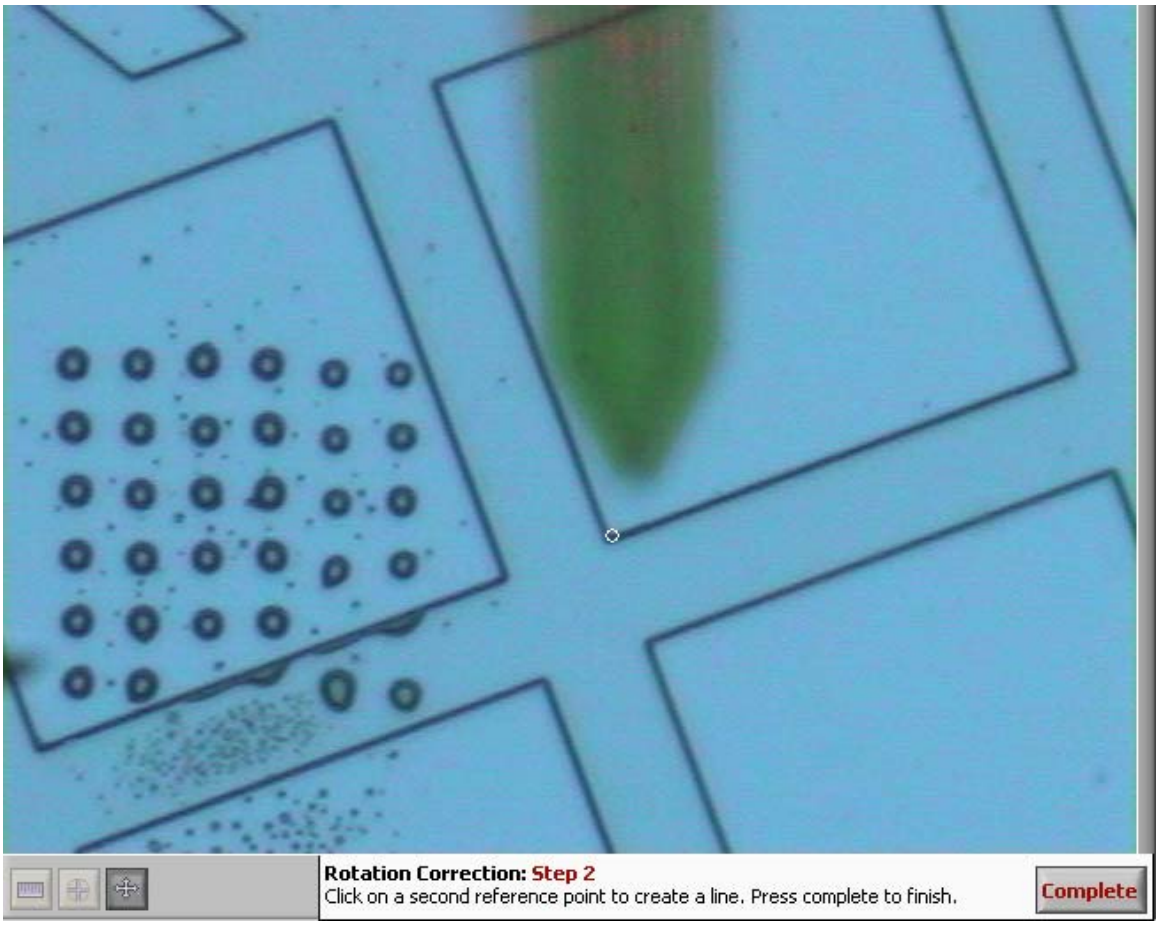
This feature allows for rotational correction on an array. For example, when using an indexed substrate such as ProLinker Sindex™ Chips, the 100 µm x100 µm square may not line up perpendicular to the X-Y stage. Therefore, the subsequent arrays would not line up properly within the square.

In order to correct the tilt, click on the SET button next to the Rotation indicator to align the SPT to the substrate. 

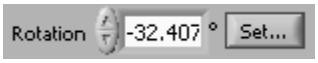
The zoom in must be set at maximum and the pointer will now show as a small circle. Place the circle on a reference line by clicking in the video screen,

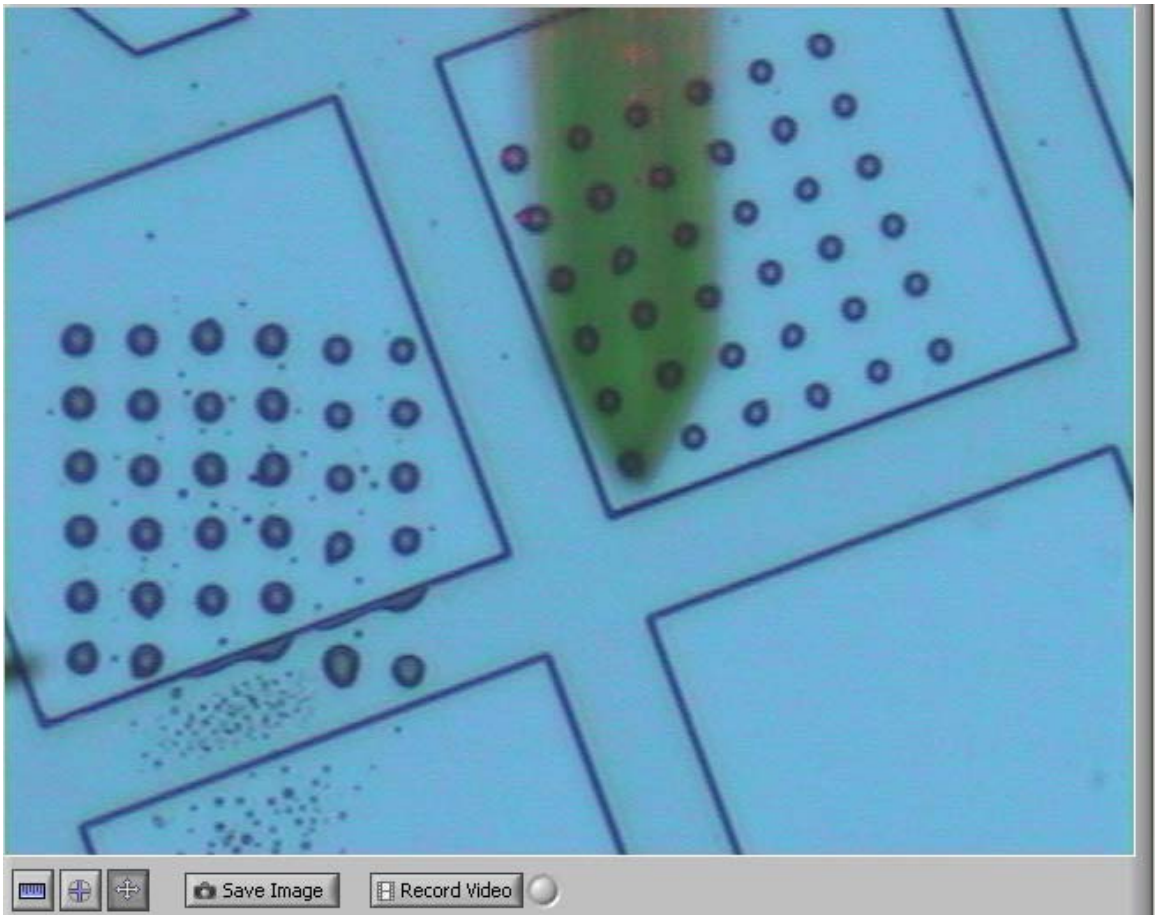


Then click on another point to create a line which the array would align to and click Complete.



The Rotation value is calculated by using the two points to create a line. All of subsequent arrays would be aligned within the 100μm x 100μm square.

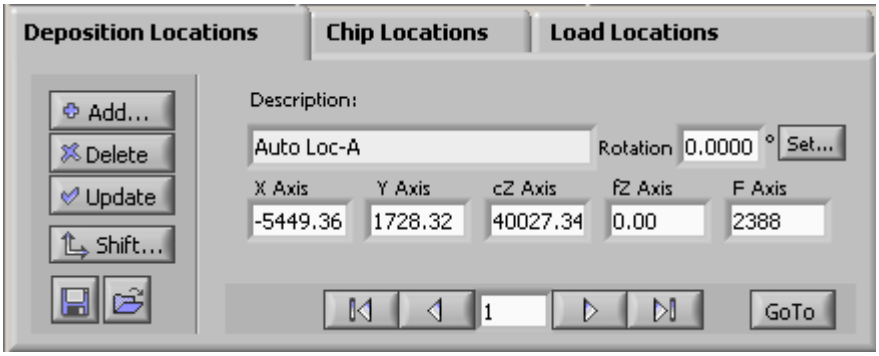




5.2 Shift Location

Shift Location allows users to construct interlaced arrays of multiple compounds with fine spatial precision. This simplifies the process by eliminating the need to realign and reposition the SPT to the substrate. For example, the user may exchange the SPT with another that is preloaded with a different compound or simply clean and reuse the same SPT.

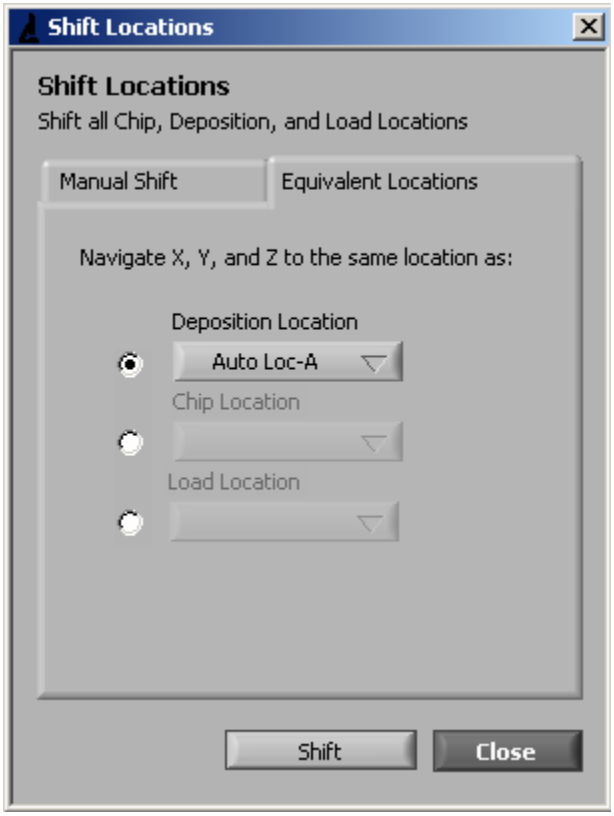
Start printing with the first SPT, making sure to save the starting position of each array or pattern as a pre-set Deposition Location. Before you remove that SPT, it should be positioned relative to a fixed reference point on the surface such that both are visible in the video window at the highest magnification. Save this reference point as a pre-set Chip Location.



Then remove the SPT and replace with your second SPT. Perform the necessary setup steps to position it in the video window, to align the laser, and to engage the surface. Navigate to the position of your reference point and position the SPT in the same manner as when the reference point was saved.

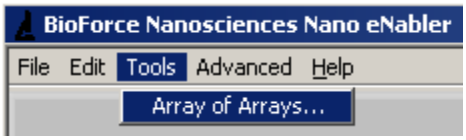
Click on the Shift button, and a window opens to confirm the equivalent location that you want to shift. 

In this case it would be the reference point that was saved as a pre-set Chip Location. This action will not physically move anything, but it does shift the coordinate values saved for each of the pre-set locations.

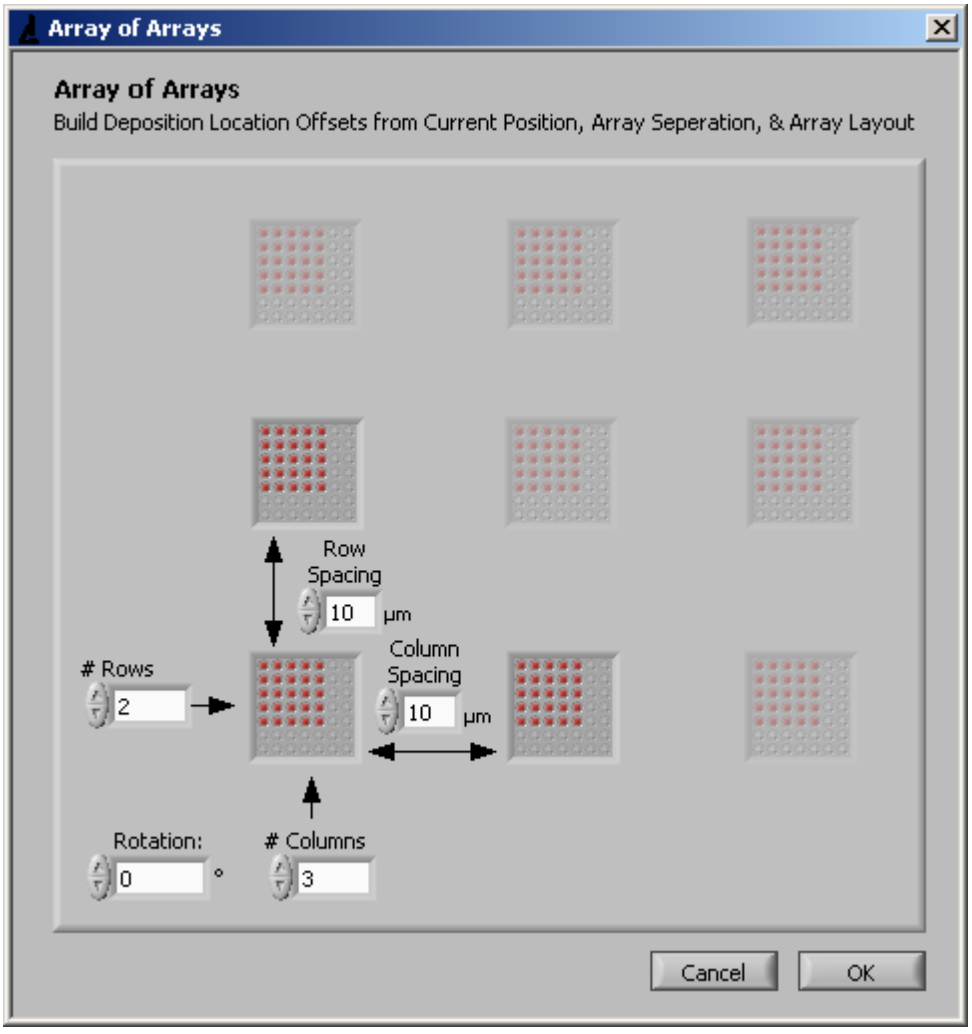


5.3 Array of Arrays

The Array of Arrays allows users to compose small multiple groupings of arrays within a larger array construct. First create a single array and go to the area of interest. Now go to tools on the drop-down menu and click on the Array of Arrays.



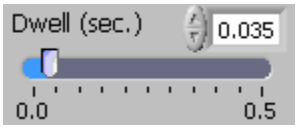
A window pops up to allow input the number arrays in rows / columns and the column / row spacing or pitch in μm. Finally click OK and GO.



5.4 On-the-Fly Arraying Features

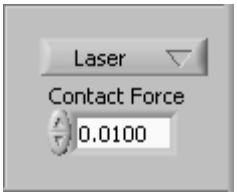
The following features are adjustable while arraying:

5.4.1 Dwell Time



5.4.2 Wait Time

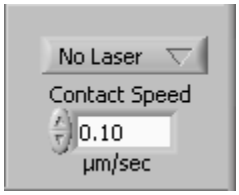
5.4.3 Contact Force



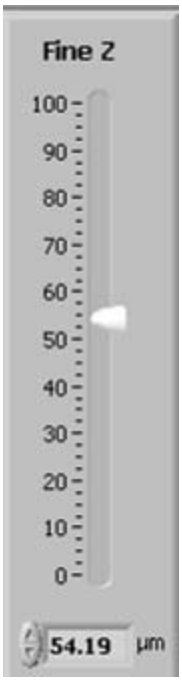
5.4.4 Withdraw Distance

5.4.5 Contact Speed

In no laser mode, the adjustment of the contact speed can be used to affect the size and shape of the spot

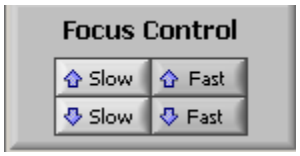


5.4.6 Fine Z



5.4.7 Illumination

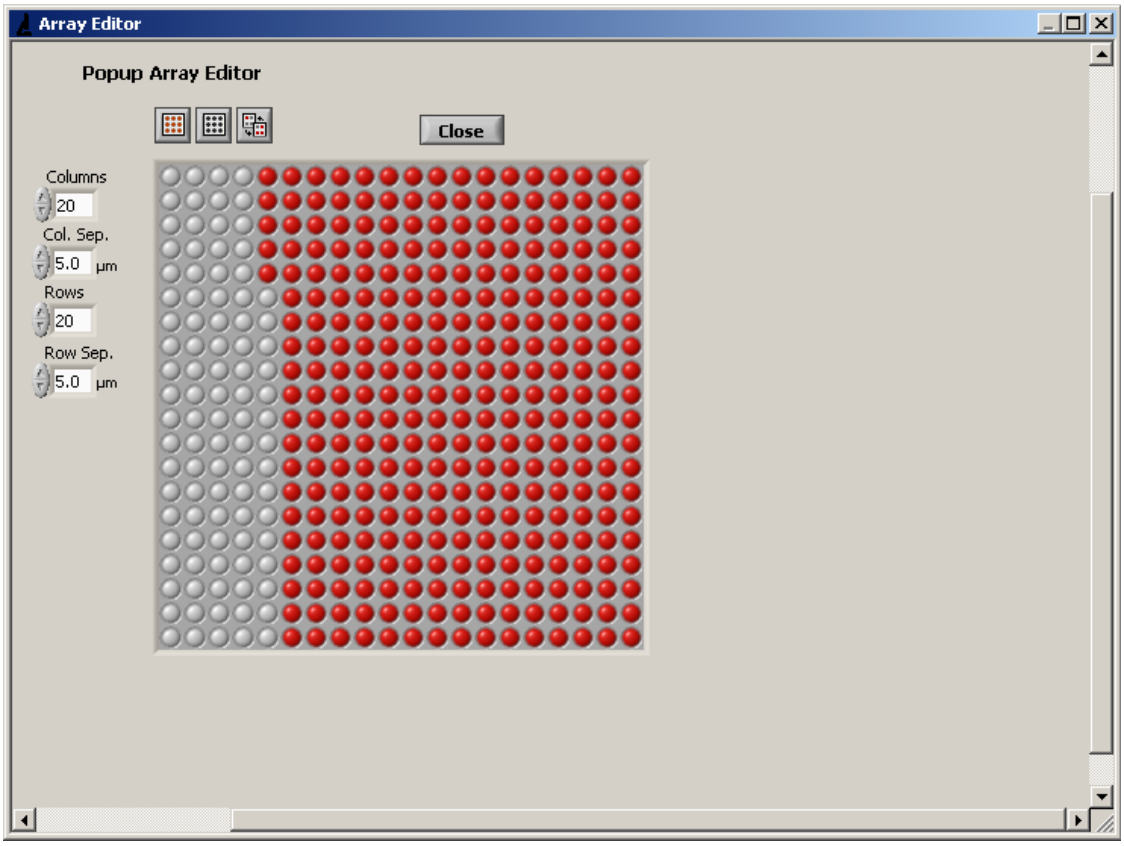
5.4.8 Focus



5.4.9 Zoom

5.4.10 Array Editor

This feature shows real-time updates of large arrays.



6 Appendix

6.1 Troubleshooting

Problems:

Why can't I find the cantilever?

If you have reached the limits of travel for the X-Y optical translation system and still cannot see the cantilever, then either the optical system is zoomed in too far, out of focus, or the cantilever is broken, positioned too far forward, too far backward, or too far to either side on the SPT holder. The cantilever focal plane depends on the length of the

lever, but is usually close to the upper limit of travel. If you still cannot find the cantilever, remove the SPT holder from the Multi-Component Head, then visually inspect and reposition the SPT.

Why doesn't the Focus Tool button work?

Due to mechanical slippage and backlash in the focusing system, the pre-set focal positions may not return to the exact focal plane at which they were set. Try refocusing the image with the fewest number of clicks possible, as each click can contribute additional error to the system. Also, attempt to capture the final focus in an upward position to reduce backlash within the stepper motor gears.

Where is the laser beam, and how do I get it positioned on the end of the cantilever?

Make sure that the laser beam has been turned on in the Nano eNabler™ software and that the green light on top of the laser itself has turned on. You should see a red line on the sample platform. Notice which direction it moves as you turn the thumbscrews. Use that knowledge to bring the laser beam onto the thick silicon substrate of the SPT or even the SPT holder. Once you can see where the focused beam is hitting, it will be easier to guide it toward the cantilever. Zoom all the way out and focus the optical microscope on the end of the cantilever. You should see the reflection of the laser beam as it strikes the cantilever.

The laser is clearly on the cantilever, so why can't I get a Sum with this SPT?

First determine the path of the laser beam. Remove the Photodetector from the Multi-Component Head to expose the aperture through which the reflected laser beam must pass. Cover this hole with a piece of paper such as a self adhesive Post-It™ note. If the laser is striking the cantilever and reflecting properly, it will show up as a red dot on the paper near the center of the hole. If the laser is skewed badly to one side of the hole or missing the hole completely, then first try adjusting the positioning of the laser. It may be slightly misaligned. If that fails, then you may need to change the angle of the cantilever in order to properly reflect the laser up to the Photodetector. Some longer cantilevers may develop warping during manufacturing, or during operation from thermal or humidity changes. In this case you can either try to position the laser further back on the cantilever to decrease the effects due to warping. This will, however reduce the sensitivity of the laser/photodetector force feedback system. If this is still not enough, you can try to change the angle of the silicon cantilever substrate itself using tiny pieces of double-sided tape as shims. Once the reflected laser beam is striking the center of the hole, replace the piece of paper with the Photodetector. If no Sum is present, check the positioning of the Photodetector as well as the wiring connections.

Why am I having problems getting the Difference set to zero?

Ensure that where the photodetector puck is oriented with the "Front" label towards the front of the device. If that doesn't help, check that the laser is centered in the photodetector hole using a small piece of semi-translucent paper (i.e.: 3M Post-it Note). Try adjusting the position of the SPT on the tool holder forward or back, and tweaking the knobs on the laser to maximize the Sum to greater than 2.

How can I tell whether or not the SPT has touched the surface?

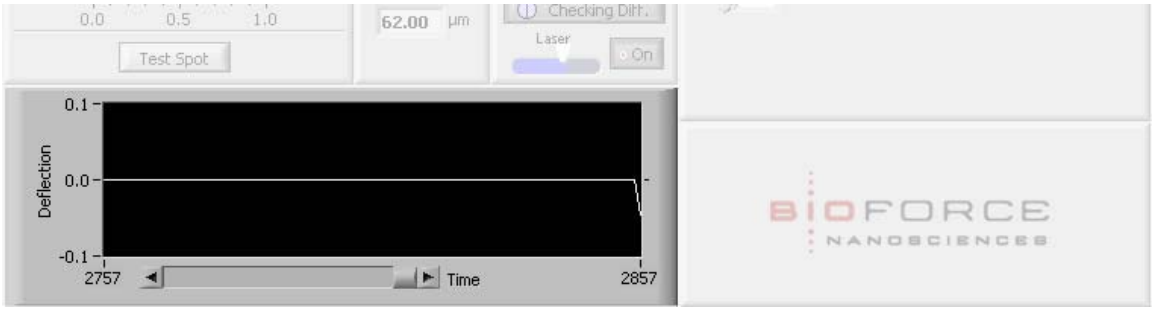
Usually there is a large jump in the deflection display on the contact force chart indicating a touch. Other indicators are large moves on the Difference meter, and watching the on-screen video for changes in the SPT color or transfer of printing material to the surface.

Should I focus on the SPT or the surface while making an array?

This is a matter of personal choice, and may depend upon your deposition settings. For longer dwell times, focusing on the SPT allows you to inspect the surface which comes into focus during the contact period. For shorter dwell times you might prefer to focus on the surface so the array is visible before and after each deposition event. Try it both ways. You can always pause an array with the Stop button, change your focal plane, and then resume the current array with the Continue button.

Why isn't the SPT touching the surface when I press Test or run an array?

Occasionally, the SPT may appear to "skip" a spot while making an array, or fail to make a Test spot. The most common cause of this problem is a contact force threshold that has been set too low relative to the noise level in the system. If the contact force threshold is set too low, a noise spike in the laser/photodetector system can be interpreted by the software as a contact event despite many microns of separation between the SPT and the surface. Observe the scrolling contact force chart to determine whether true contact was made. A real contact event will resemble a flat line that angles sharply down at the end (see example below). This malfunction can occur both in manual contact force mode and in Auto Min mode. The simplest solution is to use manual contact force mode and gently increase the contact force until the SPT touches every time. Monitor the scrolling contact force chart to judge the actual amount of force being applied.



Why is the SPT smashing into the surface?

This lack of force feedback can be the result of many different problems. First, ensure that the laser is functioning properly. Turn it on manually with the software interface and look for the green LED on the top of the laser or the focused red laser beam itself on the cantilever. Check to make sure that the laser is focused on the end of the cantilever and not the silicon substrate. If there are multiple levers on the same substrate, make sure you are watching the correct one. Raise the Coarse Z Stage until the cantilever is several millimeters above the deposition surface. This reduces any optical reflection of laser light off the surface and onto the photodetector, which might give a false Sum

value. Adjust the laser beam and photodetector positioning for maximal Sum and a Difference value of zero. With the laser turned on, click the Update button under the Noise display box. This will show the value of the minimum contact force, which is related to the amount of noise in the force feedback system. This noise level can change from cantilever to cantilever, however an excessively high noise level may signal that the cantilever is damaged to the laser is improperly positioned on the lever.

The SPT is touching the surface, but why don't I see any spots?

While it is a good indication, this observation does not necessarily mean that no materials have been transferred to the surface. Many factors can contribute to poor transfer. The SPT may have a blockage in the channel, some hydrophobic contaminants on it, the material loading may have been insufficient, the humidity may not be turned up high enough, the dwell time may be too short, or the SPT may be depleted. Try washing the SPT, and/or even removing it from the Nano eNabler™ and re-treating it in the BioForce UV/ozone TipCleaner™ for at least 30 minutes. Reload your sample, increase the humidity within the environmental control chamber, and try making depositions with longer dwell times and/or greater contact forces.

My deposition spots are too large – how can I make them smaller?

Reduce spot size by decreasing the dwell time and drying the general environment. You can also achieve smaller spots with the proper selection of an SPT design with a narrower gap at the end of the microfluidic channel.

My deposition spots are too small – how can I make them larger?

Increase spot size by increasing the dwell time and raising the humidity in the environmental chamber. You can also achieve smaller spots with the proper selection of an SPT design with a wider cantilever, channel, and gap at the end of the microfluidic channel.

How do I keep my deposition spots from merging together?

Either follow the suggestions above for reducing spot diameter, or simply increase the Col. Sep. and Row Sep. to make the spots further apart. Switching to a slightly more hydrophobic deposition surface may also help prevent spots from spreading laterally and merging together.

Why do I keep getting the dialog box reminding me to adjust the Difference, and how can I make it go away?

The Difference value has drifted too far away from zero. It is recommended that the photo sensor puck be adjusted to move the difference as close to zero as possible for maximum sensitivity.

Another option is to ignore the drifted difference value, and to toggle the button labeled "Checking Diff." so that it changes to "Ignoring Diff.". This is recommended for advanced users only.

The Stop or Cancel buttons don't seem to work - how do I interrupt a move or an array while it is in progress?

The status of the Stop or Cancel button is checked by the software when the Nano eNabler™ is at a consistent state to stop at. To effectively stop an action while it is in progress, best results can be achieved by pressing Stop button once and holding, or waiting until the software stops. Rapidly clicking the Stop buttons may actually toggle them on or off.

The General Environmental Controls don't seem to be working?

Check the supply of dry gas into the Nano eNabler™ Controller and ensure that the tank is not empty. If adequate wet and dry air is flowing into the environmental control chamber, set up your samples, surfaces, etc inside the chamber and allow the unit some time to reach equilibrium. If the Nano eNabler™ has not reached the desired humidity level in a reasonable amount of time, it may be that the desired humidity entered into the software is too far away from the ambient conditions. Sometimes the ambient conditions are too wet or too dry to achieve certain target humidity.

The* NanoWare™ *software crashed. How do I restart the software?

Perform a Ctrl+Alt+Del to bring up task manager and end task. Double click on the Nano eNabler icon to restart the program.

6.2 Quick Start Guide

Nano eNabler™ System Quick Start Guide to Printing Protein Arrays

You will need:

- Protein samples
- BioForce Protein Spotting Buffer
- SPT
- Sindex™ Chip(s)
- BioForce TipCleaner™
- Pipette (preferably 10 µl volume)
- BioForce SPT and Sindex adhesive pads

Prepare an SPT by treating with UV/ozone for 60 minutes in a BioForce TipCleaner™. Mount SPT on SPT Holder using an SPT adhesive pad from BioForce. The SPT should be positioned such that there is approximately 2 mm of overhang.

Prepare protein samples by mixing 1 µl of protein with 1 µl of BioForce Protein Spotting Buffer. Mix thoroughly. Using a P-10 pipette, aspirate approximately 0.5 µl of protein solution and dispense in the SPT reservoir. The protein solution should flow out the channel to the end of the deposition cantilever.

Turn on the power to the Nano eNabler™ System and start the software. Click on the Raise Head button in the software and then mount the SPT Holder on the Head via the magnets.

Prepare Sindex™ Chip(s) if necessary, and secure them to the Nano eNabler™ sample platform using BioForce Sindex™ adhesive pads.

Zoom all the way out and center the end of the SPT in the optical image using the X-Y adjustment knobs on the optical system.

Turn on the laser and position it halfway back along the deposition cantilever using the laser adjustment knobs. Adjust the laser position closer to the end of the deposition cantilever for greater sensitivity.

Maximize the Sum by sliding the Photodetector up and down. Zero the Difference by sliding the Photodetector forward and backward.

Close the door to the Environmental Control Chamber, activate the Environmental Controls, and set the target humidity to 50%.

Zoom in and focus on the end of the SPT. Center in the optical image again if necessary. Press the Set button to save the SPT focal plane.

Navigate to the Sindex™ Chip and lower the Head to within 3 mm of the surface. Focus down to the surface of the Sindex™ Chip and press the Set button to record the Surface focal plane.

Set the Contact Mode to Manual with a setting of 0.01, then press the Find Surface button to engage the surface.

Set deposition conditions to:

- Dwell Time to 0.0 sec (increase as necessary to make a visible spot)
- Withdraw Distance to 30 µm

Press Test to make a Test Spot. If the Fine Z Stage readout is 0.0 µm, then it isn't getting the full 30 µm of Withdraw Distance. Use the Coarse Z Stage Control (slow) to raise the head up until pressing Test gives a Fine Z Stage readout greater than 0.0 µm. Focus on the surface of the Sindex™ Chip. Design the array pattern and press Go to start patterning!

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